

DESCRIPTION

Title of Invention: DNA ANALYSIS SYSTEM

Technical Field

[0001]

The present invention relates to a DNA analysis system.

Background Art

[0002]

In fragment analysis, DNA contained in a sample is subjected to PCR using a primer designed for a specific DNA target, and fluorescently labeled DNA amplification products are separated for each size by capillary electrophoresis (CE). It is used for gene mutation analysis or quantification, cell line authentication, determination of genome editing efficiency, genotyping for amplified fragment length polymorphisms (AFLPs), simple sequence repeats (SSRs), and single nucleotide polymorphisms (SNPs), and macrosatellite marker analysis. The macrosatellite refers to repetitive DNA in which a specific DNA motif is repeated a plurality of times, and has a feature that a mutation frequency is higher and genetic diversity is higher than those of other DNA regions. As a representative example of the macrosatellite marker analysis, there is individual identification and person test using short tandem repeats (STRs). The DNA test using the STR is widely used in forensic medicine examinations, and is used for a parent-child test or a verification between DNA at a crime scene and a criminal.

[0003]

In the fragment analysis, in addition to fluorescently labeled DNA fragments to be analyzed, a plurality of types of fluorescently labeled DNA fragments having known lengths (size standards) may be mixed and subjected to CE analysis. By using the size standards, a

fragment length of each of the amplification products can be specified. In addition, if the amount of size standards to be mixed is set to a fixed amount, the amount of amplification products can be calculated based on a ratio of an intensity of the DNA fragments of the amplification products obtained by the CE analysis to an intensity of the size standards. In addition, the amount of DNA before target amplification can be estimated based on a ratio of an intensity of a target amplification product to an intensity of internal positive control (IPC) by mixing and amplifying a known amount and known length of DNA (IPC) and a primer used for amplifying the DNA in a PCR reaction solution and subjecting the same to fragment analysis.

[0004]

In a DNA test performed in the Institute of Forensic Medicine according to NPL 1, (1) a concentration of human DNA contained in a sample is quantitated by DNA quantification using quantitative PCR, (2) STR-PCR (PCR including STR sequences) is performed by preparing a sample such that the human DNA has an appropriate concentration, (3) a part of the PCR reaction solution is mixed with formamide containing size standards in a certain ratio and thermally denatured, (4) an electropherogram is obtained by subjecting the thermally denatured electrophoresis sample to a CE measurement, and (5) the DNA test is performed based on the electropherogram.

[0005]

In the DNA test or the fragment analysis using a pretreatment integrated CE analysis device of PLTs 1, 2, 3, 4, and 8, (1) a sample containing DNA is subjected to the PCR on a flow path device, (2) a part of a reaction solution is mixed with formamide containing size standards in a certain ratio and thermally denatured on the flow path device, (3) an electropherogram is obtained by subjecting the thermally denatured electrophoresis sample to the CE analysis, and (4) the DNA test or the fragment analysis is performed based on the electropherogram. Comparing PLT 1, PLT 2, PLT 3, PLT 4, and PLT 8 with NPL 1, a series

of steps is automated, and a result can be obtained in a short time (for example, 90 minutes).

[0006]

In the DNA quantification according to PLT 5, (1) in the PCR on the sample containing DNA, a part of a reaction solution at each stage of a plurality of consecutive thermal cycles n ($n = n_0, n_0 + 1, n_0 + 2, \dots$, where n_0 is a preset integer) is divided, (2) the divided reaction solutions are subjected to the CE analysis, and (3) the DNA quantification (quantification of an original concentration of DNA contained in the sample) is performed based on a relation between a peak intensity of the amplification product subjected to the CE analysis and the number of thermal cycles n , specifically, the number of thermal cycles n at which the peak intensity exceeds a predetermined threshold. That is, PLT 5 discloses an analysis method in which the amplification products are detected in real-time PCR by the CE.

[0007]

In the DNA quantification according to PLT 6, (1) in the PCR on the sample containing DNA, a part of the amplification products at each stage of the series of plurality of thermal cycles n (m is 1 or 2, and $n = n_0, n_0 + m, n_0 + 2m, \dots$, where n_0 and m are preset integers) is divided by the electrophoresis on the flow path device, (2) the divided amplification products are subjected to the CE analysis on the flow path device, and (3) the DNA quantification (quantification of the original concentration of DNA contained in the sample) is performed based on the relation between the peak intensity of the amplification product subjected to the CE analysis and the number of thermal cycles n , specifically, the number of thermal cycles n at which the peak intensity exceeds a predetermined threshold. That is, PLT 5 discloses the analysis method in which the amplification products are detected in real-time PCR by the CE.

[0008]

In the DNA quantification according to PLT 7, (1) in the PCR on the sample containing DNA, a part of the reaction solution at each stage of the plurality of thermal cycles

n is divided on the flow path device, (2) the divided reaction solutions are subjected to microarray analysis on the flow path device, and (3) the DNA quantification (quantification of the original concentration of DNA contained in the sample) is determined based on a relation between a spot intensity of the amplification product subjected to the microarray analysis and the number of thermal cycles n, specifically, the number of thermal cycles n at which the spot intensity exceeds a predetermined threshold. That is, PLT 7 discloses an analysis method in which the amplification products are analyzed in real-time PCR using a microarray.

Citation List

Patent Literature

[0009]

PTL 1: US2022/0016632

PTL 2: US Patent No. 9354199

PTL 3: US2019/0019290

PTL 4: JP2017-077180A

PTL 5: US Patent No. 7445893

PTL 6: JP5494480B

PTL 7: US Patent No. 8715924

PTL 8: US Patent No. 10767225

Non Patent Literature

[0010]

NPL 1: John M. Butler, Fundamentals of Forensic DNA Typing (2009), P.29 to 107 and P.279 to 336

Summary of Invention

Technical Problem

[0011]

In a CE analysis device or a device equipped with a CE analysis unit, a ratio between a minimum peak intensity and a maximum peak intensity at which the peak intensity and a concentration are substantially proportional is 100 or less, 1,000 or less, 10,000 or less, or 100,000 or less. When the fragment analysis is performed, a ratio of a minimum amount to a maximum amount of a measurable DNA concentration is 10, 30, 100, 300, 1,000, 3,000, or 10,000 taking into consideration of a variation in an intensity of the CE analysis device, a variation in the amount of amplicons injected into the CE, a variation in an amplification efficiency for each allele and an abundance ratio for each allele, a variation between dyes, a variation at the time of sample adjustment, and the like. On the other hand, a ratio of a minimum amount to a maximum amount of DNA contained in the sample brought into the analysis system is 30, 300, 3,000, or 30,000. When the amount of DNA contained in the sample exceeds a range of the amount of DNA contained in the sample measurable by the analysis system, the fragment analysis may fail. In this case, the sample consumed for the analysis or an analysis time is wasted.

[0012]

One object of the present invention is to subject a sample containing DNA whose concentration ratio changes in a range of 100 times or 1,000 times or more (sample concentration range is 100 or $1,000 = 2.0$, or 3.0 digits or more) to DNA test and fragment analysis or DNA quantification (quantification of an original concentration of personal DNA contained in a sample) with high accuracy and high robustness through PCR and CE analysis by a low-cost device with a simple configuration. In addition, as in PTL 2, a series of processes is automated, and a result can be obtained in a short time (for example, within 180 minutes, within 120 minutes, or within 90 minutes).

[0013]

In NPL 1, the amount of DNA brought into the PCR is quantified by the quantitative PCR so as not to exceed an analysis range, and the PCR reaction is performed by diluting the DNA to an appropriate concentration. When the present method is performed in a flow path device, an optical system for performing the quantitative PCR is necessary. In addition, a complicated flow path structure is required to determine a dilution concentration according to quantitative PCR results.

[0014]

In PLT 1, a solution is divided before the PCR, and subjected to the STR-PCR and the quantitative PCR. The amount of DNA is quantified by the quantitative PCR to determine the number of STR-PCR cycles. The solution is divided before the start of PCR, which reduces the sensitivity. In addition, a flow path device for performing two different PCRs is complicated, and an additional optical system is also required, which inevitably results in high costs.

[0015]

In PLT 2, the solution is divided before the PCR to prepare DNA solutions having two different concentrations, and both of the DNA solutions are subjected to the PCR for the DNA test. One of the two DNA solutions falls within an analysis concentration range of the analysis system, and thus the analysis range can be expanded. The solution is divided before the PCR, resulting in reduced sensitivity. In addition, a flow path mechanism for dividing a solution and adjusting a concentration is mounted on the flow path device, and thus the flow path device inevitably becomes complicated.

[0016]

In PLT 3, the analysis range of DNA test is expanded by improving a data analysis method. Information on a peak below a detection limit during the CE analysis cannot be obtained. If a detected intensity is saturated during the CE analysis, a correct peak intensity

ratio cannot be obtained. If the interpretation of the DNA test is excessively expanded, data obtained by analysis may not reflect an original mixing ratio of personal DNA. There is a risk of erroneous profiling being performed.

[0017]

In PLT 4, a part of the reaction solution is taken out after the PCR, the presence or absence and the amount of amplification products are detected by an optical system, and the fragment analysis is performed if appropriate amplification is performed. When it is determined that the amplification is not complete, the reaction solution remaining in the PCR unit is subjected to an additional PCR reaction. In the present method, it is necessary to design an additional optical system or a flow path device suitable for optical detection, which inevitably results in high costs. In addition, there is a risk that fluorescent dyes in the STR-PCR may fade during detection made by the optical system, or that optical detection may not be performed correctly due to overlap between the fluorescent dyes in the STR-PCR and a detection wavelength range of the optical system.

[0018]

In PLTs 5 and 6, it is necessary to perform the CE analysis for each of a large number of thermal cycles n (a plurality of consecutive thermal cycles n), and thus it takes a long time to obtain a result. For highly accurate quantification, three or more CE analyses are required. Although it is possible to perform the DNA quantification by replacing the PCR with the STR-PCR, a special flow path structure is required to take out a solution many times. In addition, there is no step of mixing the reaction solution and formamide containing size standards at a constant ratio, and thus the DNA test cannot be performed. In addition, when the DNA test is performed by incorporating a mixing step with the formamide (electrophoresis reagent) including the size standards into the method, a dispensing mechanism or a flow path structure for mixing a PCR product and the electrophoresis reagent in each cycle is newly required, which results in a complicated device and high costs. In addition, particularly for the DNA

test that requires the CE analysis with high accuracy and high resolution, the CE analysis on the PCR product in each cycle requires an enormous amount of analysis time. In addition, in PLT 6, DNA is taken out from a PCR chamber by applying a voltage, and thus it is expected that compositions of an amplification product in the PCR chamber are different from compositions of the amplification product taken out. In the DNA test, amplicons having different lengths are measured, but a bias depending on a length may occur when the amplification product is taken out from the PCR chamber by applying a voltage.

[0019]

In PLT 7, the DNA quantification can be performed by replacing the PCR with the STR-PCR, but it is necessary to perform the microarray analysis for each of a large number of thermal cycles n (a plurality of consecutive thermal cycles n), and thus it takes a long time to obtain a result. In addition, the CE analysis is not performed, and thus the DNA test cannot be performed.

[0020]

On the other hand, PLT 7 describes that the microarray analysis may be performed for a small number of thermal cycles n (a plurality of non-consecutive thermal cycles n), but this is not realistic. This is because, in the microarray analysis, generally, the spot intensity for the same DNA concentration varies due to a variation in a probe density and the number of probes immobilized on each spot, and a variation in a hybridization efficiency over time and space, and the presence or absence of corresponding DNA can be determined based on a strength of the spot intensity, but an accuracy of quantifying the corresponding DNA based on the spot intensity is low. That is, in order to obtain the number of thermal cycles n with high accuracy in which the spot intensity exceeds the predetermined threshold, it is necessary to perform the microarray analysis for each of a large number of thermal cycles n (the plurality of consecutive thermal cycles n). As a means for improving the accuracy of the DNA quantification made by the microarray analysis, a method of competitively hybridizing target

DNA or a target amplification product with reference DNA to the same spot is known. However, in order to perform this method, it is necessary to prepare the reference DNA for each target DNA or target amplification product that hybridizes to a probe on the spot with the same efficiency as the target DNA or the target amplification product, and further to label the target DNA or the target amplification product and the reference DNA with different fluorophores and independently measure respective emitted fluorescence. When analysis with an expanded dynamic range is implemented by preparing a small number of PCR products with different cycles and subjecting the PCR products to the microarray analysis, cost and labor are required.

[0021]

Another problem of PLT 7 is (1) in the PCR of the sample containing DNA on the flow path device, the same amount of fresh PCR solution as that of a reaction solution, which is obtained by dividing a part of the reaction solution at each stage of the plurality of thermal cycles n , is mixed with the remaining reaction solution without being divided at each stage, which changes the concentration of the DNA contained in the reaction solution and reduces the accuracy of the DNA quantification.

[0022]

Still another problem of PLT 7 is that it is necessary to repeat hybrid and dehybrid (washing) of DNA and a solid phase probe, and the solid phase probe is peeled off each time or the hybrid DNA is carried over without being washed, and thus the repetitive reproducibility of the microarray analysis is low and the accuracy of the DNA quantification is low.

[0023]

To summarize the problems of the DNA test or the fragment analysis and the DNA quantification, there is a need for a DNA analysis method that can be implemented in a simple flow path device, that can achieve low cost, high robustness, simple, sensitivity maintenance, and short measurement time, and that can widen a range of the amount of DNA to be analyzed.

Solution to Problem

[0024]

An example of a DNA analysis system according to the present invention is a DNA analysis system including:

a flow path device including a PCR chamber for performing a thermal cycle; and

a capillary electrophoresis unit configured to subject a PCR reaction solution to electrophoresis analysis, in which

the DNA analysis system

stores values of m and n set in advance,

generates a first reaction solution by subjecting the PCR reaction solution to m thermal cycles in the PCR chamber,

takes out a part of the first reaction solution from the PCR chamber without changing compositions,

subjects the part of the first reaction solution to the electrophoresis analysis in the capillary electrophoresis unit,

generates, in the PCR chamber, a second reaction solution by subjecting the first reaction solution remaining in the PCR chamber to $n - m$ (where $n - m$ is an integer of 2 or more) thermal cycles such that a total number of thermal cycles is n ,

takes out at least a part of the second reaction solution from the PCR chamber without changing compositions, and

subjects the at least part of the second reaction solution to the electrophoresis analysis in the capillary electrophoresis unit.

[0025]

An example of a DNA analysis system according to the present invention is a DNA analysis system including:

a flow path device including a PCR chamber for performing a thermal cycle; and
a capillary electrophoresis unit configured to subject a PCR reaction solution to electrophoresis analysis, in which
the DNA analysis system
stores values of m and n set in advance,
generates a first reaction solution by subjecting the PCR reaction solution to m thermal cycles in the PCR chamber,
takes out a part of the first reaction solution from the PCR chamber without changing compositions,
generates, in the PCR chamber, a second reaction solution by subjecting the first reaction solution remaining in the PCR chamber to $n - m$ (where $n - m$ is an integer of 2 or more) thermal cycles such that a total number of thermal cycles is n ,
takes out at least a part of the second reaction solution from the PCR chamber without changing compositions,
subjects one of the part of the first reaction solution and the at least part of the second reaction solution to the electrophoresis analysis in the capillary electrophoresis unit, and
controls execution of the electrophoresis analysis on another one of the part of the first reaction solution and the at least part of the second reaction solution based on a result of the electrophoresis analysis on the one of the part of the first reaction solution and the at least part of the second reaction solution.

Advantageous Effects of Invention

[0026]

Effects obtained by an example of the invention disclosed in the present application will be simply described below. That is, according to an example of the present invention,

in the analysis system including the flow path device and the electrophoresis unit, a range of the amount of DNA to be analyzed can be expanded with a small-sized device at low cost with high accuracy, high sensitivity, and a short time.

[0027]

Problems, configurations, and effects other than those described above will be clarified by description of the following embodiments.

Brief Description of Drawings

[0028]

[FIG. 1] FIG. 1 is a schematic diagram of an analysis system.

[FIG. 2] FIG. 2 is an example of a data determination method.

[FIG. 3] FIG. 3 is a schematic diagram of the analysis system.

[FIG. 4] FIG. 4 is an embodiment of the analysis system.

[FIG. 5] FIG. 5 is a schematic diagram and an embodiment of the analysis system.

[FIG. 6] FIG. 6 is a schematic diagram of the analysis system.

[FIG. 7] FIG. 7 is a schematic diagram of the analysis system.

[FIG. 8] FIG. 8 is a schematic diagram of a flow path device.

[FIG. 9] FIG. 9 is a schematic diagram of the flow path device.

[FIG. 10] FIG. 10 is a schematic diagram of the flow path device.

[FIG. 11] FIG. 11 is a schematic diagram of the flow path device.

[FIG. 12] FIG. 12 is a schematic diagram of the flow path device.

[FIG. 13] FIG. 13 is an operation example of the flow path device.

[FIG. 14] FIG. 14 is an embodiment of the analysis system.

[FIG. 15] FIG. 15 is a schematic diagram of the flow path device.

[FIG. 16] FIG. 16 is an operation example of the flow path device.

[FIG. 17] FIG. 17 is a schematic diagram of the flow path device.

[FIG. 18] FIG. 18 is a schematic diagram of the flow path device.

[FIG. 19] FIG. 19 is a schematic diagram of the flow path device.

[FIG. 20] FIG. 20 is an operation example of the flow path device.

[FIG. 21] FIG. 21 is an operation example of the flow path device.

[FIG. 22] FIG. 22 is an operation example of the flow path device.

[FIG. 23] FIG. 23 is a result illustrating a relation between an electrophoresis reagent and a peak intensity.

[FIG. 24] FIG. 24 is a diagram illustrating an effect of the present invention.

[FIG. 25] FIG. 25 is a diagram illustrating an analysis range.

[FIG. 26] FIG. 26 is an example of a table used in setting references of the number of cycles.

[FIG. 27] FIG. 27 is a schematic diagram of an obtained electropherogram.

[FIG. 28] FIG. 28 is an example of a table used in setting references of the number of cycles.

[FIG. 29] FIG. 29 is an embodiment of the analysis system.

[FIG. 30] FIG. 30 is an example of the data determination method.

[FIG. 31] FIG. 31 is an example of the data determination method.

[FIG. 32] FIG. 32 is an embodiment of the analysis system.

[FIG. 33] FIG. 33 is an embodiment of the analysis system.

Description of Embodiments

[0029]

In the present specification, procedures or references for performing human DNA test are mainly described, but an analysis target is not limited to the human DNA test.

[0030]

Hereinafter, unless otherwise specified, m and n indicate the number of thermal

cycles of PCR. m and n may be integers, and $n - m \geq 2$. Hereinafter, a thermal cycle may be referred to as a PCR cycle or simply a cycle.

[0031]

Hereinafter, a solution obtained by mixing a PCR reaction reagent and sample-derived DNA is referred to as a PCR reaction solution. Among the sample-derived DNA, DNA amplified by the PCR reaction reagent is referred to as target DNA.

[0032]

Hereinafter, the PCR reaction solution obtained after m thermal cycles is referred to as a "PCR reaction solution m ", and the PCR reaction solution obtained after n thermal cycles is referred to as a "PCR reaction solution n ".

[0033]

Hereinafter, an electrophoresis reagent may include deionized formamide, size standards, and pure water. The formamide or pure water may be contained for decreasing an ion intensity of an electrophoresis sample or for denaturing DNA. Not only formamide or pure water but also a solution having a low conductivity may be used as the electrophoresis reagent. The solution having a low conductivity preferably has a conductivity of 10 mS/cm or less, more preferably 1 mS/cm or less, more preferably 100 μ S/cm or less, and more preferably 10 μ S/cm or less. As the conductivity of the solution used in the electrophoresis reagent is lower, the amount of DNA injected into CE tends to increase. The size standards may be mixed to associate a detected peak with a DNA length, or may be mixed to estimate the amount of DNA contained in the electrophoresis sample based on the detected peak.

[0034]

In this way, one example of an analysis system generates a mixed solution by mixing the PCR reaction solution (for example, at least one of a part of the PCR reaction solution m and at least a part of the PCR reaction solution n) with pure water or formamide or a solution having a conductivity of 10 mS/cm or less before electrophoresis analysis. A peak intensity

of CE can be increased by mixing with the solution having a low conductivity. In addition, DNA can be denatured by mixing with the formamide.

[0035]

Hereinafter, DNA obtained by the PCR reaction is referred to as an "amplification product", an amplification product obtained in m cycles is referred to as an "amplification product m", and an amplification product obtained in n cycles is referred to as an "amplification product n".

[0036]

Hereinafter, the mixed solution of the PCR reaction solution and the electrophoresis reagent is referred to as the "electrophoresis sample". When the electrophoresis sample is prepared, if the sample is heated to 90°C or higher as illustrated in (2) of FIG. 5, DNA is likely to be denatured to form a single strand, and thus more accurate CE analysis can be performed, and it is preferred to add a heating step. The sample indicated by the "electrophoresis sample" may be a sample before or after being denatured by heating. The electrophoresis sample prepared by the amplification product m is referred to as an "electrophoresis sample m", and the electrophoresis sample prepared by the amplification product n is referred to as an "electrophoresis sample n".

[0037]

Hereinafter, a PCR reaction procedure in which m PCR cycles are performed, the part of the PCR reaction solution m is taken out, and the remaining PCR reaction solution m is subjected to n - m PCR cycles, and finally both the PCR reaction solutions m and n are prepared, is referred to as "split PCR".

[0038]

Hereinafter, a locus refers to a location of a gene on a chromosome. A typical kit for STR-PCR includes primers that can uniquely amplify each locus.

[0039]

Hereinafter, alleles refer to genetic variants that can be distinguished at the same locus. At the time of DNA test, if the DNA comes from one person, there may be two alleles at the same locus (heterozygote) or one allele (homozygote).

[0040]

Hereinafter, an amplicon refers to an amplification product having a single length. A single allele may produce amplicons having different lengths. For example, a plurality of amplicons are caused by by-products (artifacts) generated in the PCR reaction. In the DNA test, a peak of one amplicon per allele is assigned to personal DNA, but in the case of mixed samples, it is difficult to distinguish between artifacts and allele-derived amplicons, and thus peaks of the amplicons that may be the artifacts may also be subjected to analysis.

[0041]

Hereinafter, the CE analysis refers to a series of flows including preparing an electrophoresis sample, subjecting the electrophoresis sample to a CE measurement, obtaining an electropherogram, and performing the DNA test or fragment analysis. However, a range indicated by the "CE analysis" does not necessarily include a part of the above flows.

[0042]

Hereinafter, the electropherogram refers to a diagram obtained by performing the CE measurement, in which a horizontal axis represents time, measurement point, or DNA strand length, and a vertical axis represents intensity. The vertical axis represents wavelength and may be three-dimensional data including intensity information. In addition, the vertical axis represents intensity and may be three-dimensional data including dye information. The electropherogram obtained from the electrophoresis sample m is referred to as an "electropherogram m", and the electropherogram obtained from the electrophoresis sample n is referred to as an "electropherogram n".

[0043]

Hereinafter, a DNA profile refers to a DNA type obtained by analyzing an

electropherogram, a two-dimensional sequence including peak intensities and peak lengths, or a data set including the number of repetitions and peak intensities of DNA assigned to the DNA type.

[0044]

Hereinafter, STR-CE refers to a series of flows including preparing a PCR reaction solution of STR-PCR, performing a PCR reaction, performing CE measurement, and analyzing the obtained electropherogram. The data obtained from the STR-CE may be an electropherogram or a DNA profile. At a stage in which the STR-CE is completed, a part or the whole of the data may or may not be provided to a user.

[0045]

Hereinafter, an analysis range refers to, for example, a range of the amount of given samples or the quantity of given biomolecules in which the given samples or the given biomolecules can be correctly analyzed for an analysis system, or a part or a plurality of execution procedures included in the analysis system. Here, the expression "can be correctly analyzed" may refer to a state in which all required conditions are satisfied, but it is not necessary to satisfy all the required conditions, and the expression "can be correctly analyzed" may also refer to a state in which data in the best state that can be implemented in the system can be provided. For example, when the amount of given samples is extremely small, the obtained data cannot satisfy all the required conditions, but data closest to satisfy the required conditions may be obtained.

[0046]

The PCR reaction solution may contain two or more primer sets, and the PCR reaction solution may contain two or more amplified gene regions. By analyzing a plurality of amplified gene regions, the personal or individual identification capability is enhanced. In particular, in a case of the person test, it is possible to reduce a risk of being erroneously determined to be the same person. The number of PCR chambers can be minimized by

subjecting the plurality of gene regions to PCR reactions at once instead of individual PCR reactions. The reaction reagent is saved. A risk of lowering the sensitivity can be avoided by dividing the PCR chambers.

[0047]

Embodiment 1.

[0048]

[Analysis System]

In the present embodiment, an analysis system 101 (DNA analysis system) may include a memory that stores a program instruction, a control unit including a processor that executes the program instruction, a function of receiving raw data, optical data, and electropherogram data from a detection unit and analyzing these data, a solution transport control mechanism such as a pump or a valve, a CE unit (capillary electrophoresis unit) that subjects the PCR reaction solution to the electrophoresis analysis, a flow path device, and a heater. A control analysis unit may be connected to a network, and may upload, match, and access data from a personal DNA database. The control analysis unit may be connectable to, for example, a combined DNA index system (CODIS). The pump may be a diaphragm pump or a syringe pump. As an example of the valve, a valve that directly or indirectly transmits motor power and deforms a film or a valve that deforms the film using an air pressure may be used. The valve may be controlled by the control unit. The valve may be opened and closed by being deformed using heat, or using a magnetic force.

[0049]

Various parameters related to an analysis protocol may be stored in a database of the computer 102 provided in the analysis system 101 in advance. The computer may play a role of opening and closing valves of a flow path device 104, a CE unit 105, a connection portion thereof, and the like, performing temperature control, and controlling an applied pressure and/or a flow rate based on parameters recorded in the database 103. The

parameters recorded in the computer 102 may store a function for setting the parameters based on the temperature, the time, the pressure, the flow rate, the stored parameters, and measured values.

[0050]

A sample containing target DNA may be received, and dissolution, purification, PCR, and CE measurement and analysis may be fully automatically performed. A part of dissolution, purification, PCR, and CE measurement and analysis may be fully automatically performed. For example, the analysis system 101 or the flow path device 104 fully automatically performs a process illustrated in FIG. 14, which will be described later, from preparing the PCR reaction solution to subjecting the PCR reaction solution to n - m thermal cycles. In this way, the process is performed more efficiently. The analysis becomes possible by non-experts.

[0051]

The dissolution, purification, and preparation of the PCR reaction solution may be performed in an integrated device. The PCR and the formamide may be mixed and heated in the integrated device. The CE measurement may be performed in the integrated device.

[0052]

The flow path device 104 may be disposable. The contamination between samples can be prevented by making the flow path device 104 disposable.

[0053]

The CE unit 105 may be disposable. The contamination between samples can be prevented by making the CE unit 105 disposable. In addition, it can be molded integrally with the device, and thus the storage, maintenance, and transportation are facilitated. A connection portion between a pretreatment unit and the CE unit becomes simple, and a frequency of failures or errors can be reduced.

[0054]

While a pretreatment flow path device is disposable, the CE unit may be usable a plurality of times. The CE unit is required to be precisely manufactured, and has a high unit price, and thus the cost can be reduced by making the CE unit disposable.

[0055]

FIG. 1 illustrates a detailed example of the analysis system 101 and the computer 102.

[0056]

The computer 102 may include a user interface 106. Parameters related to the user interface 106 (for example, time, temperature, pressure, flow rate, procedure, divided liquid amount, PCR cycle number, sample information, cartridge information, analysis protocol, and the like of each step) may be received from the user and stored in the database 103. In addition, the various parameters may be stored in the database 103 in advance. The computer 102 may play a role of opening and closing the valves of the flow path device 104, performing temperature control, and controlling the applied pressure and flow rate, based on the parameters recorded in the database 103.

[0057]

The flow path device 104, which is consumed for each measurement, may include a tag inside, and the analysis system 101 may read information on the tag to set an appropriate analysis protocol.

[0058]

The number of PCR cycles (for example, values of m and n) may be set by the user. The analysis system 101 stores the values of m and n set in advance. In addition, the user may input information related to the type of sample (for example, cheek swab/touch sample/casework sample/DVI), and may determine appropriate PCR execution procedures by collating with a database in the computer. When the electrophoresis is performed a plurality of times or on a plurality of electrophoresis samples, the user may select whether to subject the electrophoresis sample m to the CE measurement first or subject the electrophoresis

sample n to the CE measurement first by input. In addition, all of the execution procedures may be automatically controlled by the computer 102. In addition, the user may assist and perform a part of an analysis flow.

[0059]

In the related art, a flow was general in which a sample is sent to an environment in which experimental equipment is arranged as in an institute or the like, an examiner having specialized knowledge and technique performs sample adjustment and measurement, and performs data analysis. However, problems include a time it takes to transport samples and a large amount of equipment cost and labor cost required to maintain the experimental equipment. In addition, when batch processing is introduced to increase efficiency, it is difficult to insert urgent samples. In recent years, sample-to-answer (StoA) systems, which fully automatically perform a process from sample introduction to measurement and data acquisition, appear in various fields. A flow path device in which a chamber, a flow path, and a reagent are integrated may be used in the StoA system. The introduction of the flow path device has the following advantages. (1) The measurement can also be easily performed by the non-experts, (2) data can be acquired in a short time, (3) a highly portable device can be designed, (4) variation due to a procedure can be reduced, and (5) a reagent can be easily stored.

[0060]

The potential field of application of the StoA system includes, for example, forensic medicine, DNA test, extracorporeal diagnosis, identification of species of animal or plant, biodefense, medicine, biotechnology, life science, defense, public hygiene, and agricultural. The StoA system may be used in institutes, crime scenes, police stations, hospitals, automobiles, and the like.

[0061]

[Flow Path Device]

In the present embodiment, the flow path device 104 refers to a disposable or multiple-usable cartridge that includes a reagent, a chamber, and a flow path inside. The flow path device 104 may include a power source for transporting a solution therein. In addition, a part or all of the reagents may be present within the device. A part of the chamber may be provided with a temperature control function, a molecular capture function, a detection function, and a voltage application function.

[0062]

A material used for the flow path device is not particularly limited as long as it is a material generally used in the technical field. It is desirable to use, as materials that have a low DNA adsorption amount, polypropylene, cyclic olefin polymer (COP), cyclic olefin copolymer (COC), polycarbonate, polyethylene terephthalate, and polyurethane. It is also desirable to reduce the adsorption amount by modifying a surface to be negatively charged. Examples of other materials include, for example:

- metals such as gold, silver, copper, aluminum, tungsten, molybdenum, chromium, platinum, titanium, and nickel;

- alloys such as stainless steel, Hastelloy, Inconel, Monel, and duralumin;

- silicon;

- glass materials such as glass, quartz glass, fused silica, synthetic quartz, alumina, sapphire, ceramics, forsterite, and photosensitive glass;

- plastics such as polyester resin, polystyrene, polyethylene resin, acrylonitrile butadiene styrene resin (ABS resin), dimethylpolysiloxane (PDMS), nylon, acrylic resin, fluororesin, polycarbonate resin, polyurethane resin, methyl pentene resin, phenolic resin, melamine resin, epoxy resin, and vinyl chloride resin;

- agarose, dextran, cellulose, polyvinyl alcohol, nitrocellulose, chitin, and chitosan, or any combination thereof.

[0063]

[Chamber and Reagent Storage Unit]

A typical chamber or reagent storage unit refers to a space in which a liquid or a solid can be stored and a solution can be reacted, left to stand, heated, or changed. The chamber may have a diameter larger than that of the flow path, but may not be distinguished from the flow path by eyes. The chamber may have a film or a fine structure inside, may have compositions different from those of the flow path, may have a different surface treatment, or may have a different hydrophilicity. In addition, a heater or a laser light source may be attached to the outside of the flow path device. The reagent may be stored in the chamber, or the PCR, dissolution, purification, and the like may be performed in the chamber. A typical volume of the chamber is preferably 0.01 μL to 50 mL.

[0064]

The flow path device may store the reagent in the device, or may be supplied with the reagent from the outside of the flow path device or from the inside of the analysis system. As an example, one or more kinds of reagents are stored in one or more reagent storage units in the device. The reagent contains at least one or more of [a solution, a washing liquid, a PCR reagent (which may include a polymerase, a primer, and a surfactant), formamide, pure water, a DNA fragment, and oil]. These reagents are mixed at an unintended timing, which can result in reduced performance or other unexpected results, and thus it is desirable to separate these reagents until just before use by a barrier mechanism including a valve, film, air, or a flow path that is thin enough to prevent spontaneous mixing, or a combination of these. In addition, by isolating the reagents from outside air, the long-term storage and the device portability are achieved. The same reagent may be stored in a plurality of reagent storage units for discharge in a plurality of steps. Similarly, when a reagent is stored outside the device, the reagent is preferably stored in a state separated from the outside air, and is separated from other purification system components by a valve, a film, air, or the like. Known reagent storage techniques include, for example, a blister reagent storage unit or the

reagent storage units described in PTLs 1 and 2, and a similar form may be incorporated into the present embodiment.

[0065]

[Sample Type]

The sample to be supplied to a purification system according to the present embodiment is not particularly limited as long as it is a biological sample. The biological sample is also not particularly limited, and any biological sample such as vertebrates (for example, mammals, birds, reptiles, fishes, and amphibians), invertebrates (for example, insects, nematodes, and crustaceans), plants, protists, fungi, bacteria, and viruses can be used.

[0066]

When the sample is collected, a swab, filter paper, cloth, and the like can be used as a carrier, and the carrier may be introduced into the purification system.

[0067]

A forensic medicine sample includes a cheek swab, a bone, a muscle tissue, a human organ, a sample containing a very small amount of DNA called a touch sample, a blood stain, a skin piece, hair, a body fluid, and a corpse product that is estimated to have these samples attached. Many forensic medicine samples contain an unknown amount of DNA, and the DNA has a concentration of 0.001 ng to 1,000 μ g, and more frequently has a concentration of 0.01 ng to 10 μ g. In addition, the forensic medicine sample may contain only DNA from a single person, may contain DNA from a plurality of persons, and may contain deteriorated DNA.

[0068]

[Solution Transport Control]

The analysis system 101 may include a pump or a valve for transporting a solution. As a transport means, a syringe pump, a diaphragm pump, an electrochemical pump, passive transport using a surface tension, centrifugal force, and a combination thereof may be used.

[0069]

The analysis system 101 may include a valve. The valve is used to specify a solution transport path and to switch a path through which the air pressure is applied. As the valve, a diaphragm valve operated by the air pressure, a mechanical valve, or a valve using a surface tension may be used. The valve may switch the flow path through which a solution can be transported depending on a difference in pressure required for transport.

[0070]

[PCR Reaction]

The device may include PCR reagents. The PCR reagent may separately include a solution containing a polymerase and a solution containing a primer. The PCR reagent may be a dry reagent. The sample itself, such as a swab, can also be supplied to the PCR. The DNA purified with silica or purified with Chelex or phenol chloroform may be mixed with a PCR reagent. A DNA-trapped film (silica film or the like) may be mixed with the PCR reagent.

[0071]

The PCR reagent may include a set of an IPC amplified together with the sample DNA and a primer for amplifying the IPC. The primer for IPC has a dye attached thereto, and may be detectable by CE. Amplicons derived from the IPC can be used for analysis. The amount of DNA of the sample may be estimated by using an intensity ratio of an IPC-derived peak to a sample-derived peak, an amplification efficiency correction coefficient, and a fluorescence intensity correction coefficient. In addition, by confirming the intensity of the IPC, it is possible to estimate whether the PCR reaction is proceeding normally or is inhibited.

[0072]

A typical amount of the PCR reaction solution is 1 μ l to 200 μ l, more preferably 10 μ l to 50 μ l. When the amount of solution is small, there are advantages that accurate

temperature control is possible, high-speed PCR is possible, and reagent cost is low. On the other hand, if the amount of solution is large, more eluted DNA can be received.

[0073]

A typical PCR reaction may include an initial denaturing step, an annealing step, an extension step, a denature step, and a final extension step, and some steps may be omitted.

[0074]

In the initial denaturing step, the PCR reaction can be started by being heated at 90°C to 99°C for 1 s to 2 min at the start of the PCR. In the annealing step, the primer is bound to the template DNA by being heated at 50°C to 80°C for 1 s to 2 min. In the extension step, a DNA extension reaction is performed by being heated at 50°C to 80°C for 1 s to 2 min and raising the temperature to a temperature at which a DNA polymerase can function effectively. In the denature step, heating is performed at 80°C to 99°C for 1 s to 2 min. In the final extension step, heating is performed at 50°C to 80°C for 1 min to 60 min. By providing the final extension step, lengths of the amplification products can be made uniform. The three steps, that is, the annealing step, the extension step, and the denature step are repeated 10 to 40 times. The annealing step and the extension step may be performed at the same temperature.

[0075]

[Detection Method]

After the amplification, the detection by the CE is performed. In the CE, a method of injecting amplification products into a capillary filled with a polymer by voltage injection may be used. Further, when a high voltage is applied to both ends of the capillary, DNA fragments emitting fluorescence are separated for each size and detected by a laser or a camera system. Although only the CE analysis is described in the present embodiment, instead of the CE unit, in another embodiment, a massively parallel sequencing (MPS), pyrosequencing, sanger sequencing, nanopore sequencing, chromatography, electrical measurement,

spectroscopy, NMR, restriction fragment length polymorphisms (RFLP), a microarray, and the like may be used.

[0076]

[Analysis and Peak Determination References]

A signal obtained in the CE unit is analyzed in the analysis unit. Known analysis software includes GeneMapper (registered trademark) ID, GeneMapper ID-X, GeneMarker (registered trademark) HID, i-Cubed (trademark), OSIRIS, TrueAllele (trademark), and the like. In the CE analysis, a diagram in which a horizontal axis represents a DNA length and a vertical axis represents an intensity is generated based on a size standard peak from the information on a signal intensity vs time at the time of analysis. Cosmic rays or pull-up and pull-down may be corrected. There may be baseline correction, and electropherograms may be obtained by using other known techniques. The obtained electropherograms are subjected to peak detection to examine an intensity and a peak position of each amplicon. The analysis may be partially performed with human intervention or may be performed fully automatically.

[0077]

In this section [Analysis and Peak Determination References], the peak determination references related to the DNA test will be described. The similarity determination references may be used in another fragment analysis.

[0078]

The macrosatellite refers to a DNA locus containing a repetitive nucleotide sequence having 2 to 7 nucleotides per repeat unit. The number of repeats of a specific locus varies for each individual, and can be detected as a difference in the lengths of the amplification products by the STR-PCR.

[0079]

In a typical STR-PCR analysis, two or more loci are detected. Typically, 5 or more, 10 or more, 15 or more, 20 or more, or 25 or more loci are included. The STR-PCR may be

commercially available kits such as GlobalFiler (trademark) and Power Plex (registered trademark). In addition, it is also preferred that the loci include loci for a forensic medicine or DNA test in various countries, such as CODIS, or loci designated by various gene databases. The loci may include a locus present on the normal chromosome or may include a locus present only on the Y gene.

[0080]

Specific loci may be a mixture of homozygous or heterozygous, and the number of peaks detected when the DNA type derived from a single person is examined corresponds, at minimum, to the number of loci of the kit except for a case in which the amount of DNA is deteriorated or insufficient, and corresponds, at maximum, to a peak corresponding to a sum of the double amount of the genes assigned to the euchromosome and the number of the genes assigned to the chromosome among the loci of the kit.

[0081]

In the typical STR-PCR analysis, one fluorescent dye is assigned to one locus. STR-PCR kits using 2, 3, 4, 5, 6, 7, or 8 dyes may be used. When DNA types are detected, the loci can be assigned based on a combination of information on length and peak color. Thresholds related to intensities or positions of various peaks may be set for each color, for each locus, or for each allele.

[0082]

In a DNA profile derived from a single person, one or two alleles per locus can be detected. On the other hand, in the DNA profile of the mixed sample derived from a plurality of persons, one, two, three, or four or more alleles per locus are detected. When a personal DNA profile is assigned from the DNA profile derived from the plurality of persons, the DNA profile is typically analyzed probabilistically based on the peak intensity ratio. Examples of programs for analyzing a mixed sample include Kongho, LikeLTD, LRMix, STRmix, Euroformix, and TrueAllele.

[0083]

As an example, the artifact in the DNA test refers to a peak not derived from a personal DNA type, a balance between peaks different from an ideal state, or a peak shape different from the ideal state, which causes a difference in an electropherogram or a DNA profile obtained when the DNA analysis is actually performed on an ideal electropherogram or a DNA profile to be acquired from the personal DNA type.

[0084]

There are a plurality of mechanisms involved in the generation of artifacts. When DNA contained in the sample is derived from a single person, the presence of some artifacts is allowed.

[0085]

On the other hand, when there are many artifacts, a mechanical determination accuracy decreases, and thus a review made by experts is required.

[0086]

In addition, if there are many artifacts, erroneous DNA test results may occur.

[0087]

In addition, if the artifact is large, the DNA type to be acquired cannot be detected, and the amount of information to be acquired may be reduced.

[0088]

In addition, in a case of a sample that cannot be guaranteed to be derived from DNA of one person, typically, a sample in which a possibility that a plurality of persons are involved cannot be excluded, such as the forensic medicine sample, when there is an artifact, the analysis of the electropherogram becomes more complicated or difficult. For example, when three or more peaks appear at a single locus, it becomes difficult to interpret whether the peaks are derived from artifacts or two or more persons. In addition, a personal DNA profile is generally assigned based on the peak intensity ratio, but the assignment becomes difficult

when the possibility of artifacts is taken into consideration. Therefore, it is desirable to acquire electropherograms having as few artifacts as possible.

[0089]

A state when a peak not derived from a personal DNA occurs during the CE analysis and is reflected in an analysis result is referred to as "drop in". In addition, a state in which a peak that should be present in the personal DNA is not detected during the CE analysis and is not reflected in the analysis result is referred to as "drop out". It is preferred to set various thresholds such that drop in and drop out do not occur as much as possible.

[0090]

<Stutter Peak>

Stutter peaks are by-products of the PCR amplification. This is because one or more repeated sequences are skipped or redundantly amplified during the extension reaction. The stutter peaks typically appear before and after the sample-derived peak, and appear one or two repeats more than or less than the sample-derived peak. Typically, the stutter peak has an intensity of about 1% to 20% of the sample-derived peak.

[0091]

<Adenyl Added Uncomplete>

In the STR-PCR, extra adenyl groups are added to an amplicon having a correct length at a constant probability during the extension reaction (A⁺ peak). In a typical STR-PCR kit, a reaction step called "final extension" is added at the end of the PCR reaction. In the final extension step, adenyl groups are added to an amplicon to which no extra adenyl group is added. By providing a sufficient final extension time, a state in which adenyl groups are added to almost all the amplicons is implemented. However, if the final extension time is set too long with respect to the amount of amplicons, a proportion of the amplicons (A⁺⁺ peak) to which the adenyl groups are additionally added increases. The A⁺⁺ peak is detected at a position one base longer than the A⁺ peak. In addition, when the amount of amplicons

is excessive, the addition of the adenyl groups is not completed within the final extension time, and the amplicons to which no adenyl group is added remain and are detected as A- peaks that are less than one base. Hereinafter, the A++ peak and the A- peak are collectively referred to as an incomplete adenylation peak (IAP). In addition, when only the A- peak is described, the A- peak is written as "IAP-", and when only the A++ peak or a peak to which more adenyl groups are added is described, the A++ peak or the peak is written as "IAP+". In a preferred STR-CE, various PCR parameters such as the number of PCR cycles, the final extension time, and the amount of DNA input are adjusted such that the A- peak and the A++ peak are respectively within a range of 50% or less, preferably 20% or less, and more preferably 10% or less with respect to the A+ peak intensity. An IAP intensity threshold or an incomplete adenylation peak ratio threshold (IAPT) with respect to a main peak may be provided for determining whether the intensity of the A- peak or the intensity of the A++ peak falls within the above range and the preferred STR-CE can be performed. When an intensity ratio of the A- peak or the A++ peak to the A+ peak is a certain value or more, the intensity of the main peak does not reflect an original abundance ratio of the gene. In addition, in the case of the mixed sample, or in the case in which a peak shifted by one base appears due to genetic polymorphism, accurate assignment cannot be performed. It may be a cause of detecting an erroneous DNA type.

[0092]

<Peak Intensity Ratio>

When there is a sufficient amount of DNA, two peaks derived from the heterojunction loci show substantially the same height. When the amount of DNA is insufficient, a probability that the amount of DNA derived from each gene becomes non-uniform increases, and a difference between the two peak intensities becomes significantly large. In addition, when amplification is excessive, short DNA is amplified preferentially to long DNA. Heights of peaks derived from the same locus also differ greatly because the short DNA is

preferentially amplified. When a ratio of two peak intensities is increased, it is difficult to distinguish the peaks from the stutter peak. In addition, it becomes difficult to assign DNA in the mixed sample. Therefore, to determine whether significant CE analysis is performed, a reference is that a ratio of an intensity of a small peak to an intensity of a large peak (peak to height ratio, PHR) among the two peaks is 10% or more, preferably 40% or more, and more preferably 60% or more.

[0093]

<Ski-slope>

When the amount of DNA is excessive, an abundance ratio of dNTP or Polymerase to the amplicon decreases, and a tendency for the short DNA to be preferentially amplified increases. In this case, a slope electropherogram having a small peak of the long DNA and a large peak of the short DNA is obtained. In addition, when DNA is deteriorated, an abundance ratio of the short DNA tends to be larger than that of the long DNA. At this time, a DNA profile having a slope is also obtained. In addition, when an inhibitor is contained, an amplification efficiency of the long DNA tends to be smaller than that of the short DNA, which also provides a DNA profile having a slope. In addition, when the PCR reagent is diluted with a DNA solution at a mixing ratio larger than an original mixing ratio, a difference in the amplification efficiency may occur, and the long DNA may be preferentially amplified to provide a DNA profile having a reverse slope. In addition, in a reaction system in which a large amount of primers for the long DNA are mixed into the PCR reagent and the long DNA is preferentially amplified, a DNA profile having a reverse slope is obtained. In the DNA test, the profile having a slope or a profile having a large peak intensity ratio between the loci is not preferable. This is because the difference in the peak intensities becomes large, and a peak that saturates the CE or becomes equal to or less than a detection lower limit more likely to appear. In addition, this is also because it is difficult to assign DNA in the mixed sample. Typically, it is desirable to adjust PCR reaction parameters such that the peak intensity ratio

between the loci (inter locus PHR) is such that a minimum peak has an intensity of 1% or more, preferably 5% or more, more preferably 10% or more, and still more preferably 20% or more with respect to a maximum peak. In addition, small peaks that do not satisfy a threshold of the inter locus PHR may be excluded from peak analysis. In addition, to deal with the deteriorated DNA, it is desirable that the amount of amplification of PCR is within an appropriate range with respect to STR-CE or that a dynamic range of CE is designed to be large.

[0094]

<Saturation of CE>

If the amount of amplicons introduced into the CE unit is excessive and a fluorescence intensity at the time of CE detection exceeds an upper limit of a detection range of a detector, the saturation occurs. At the time, a ratio of a maximum intensity of the peak to the other peak intensities does not reflect the original intensity ratio. In addition, the intensity ratio of the stutter peaks or the main peaks of the IAP is detected larger than a ratio of original amplicons. Therefore, the artifact described above is highlighted. When a sample is Mix, a correct mixing ratio cannot be calculated. Therefore, in a preferred DNA test, PCR reaction conditions are set or PCR amplification products are diluted such that the CE detection does not show oversaturation (OS). An OS threshold may be evaluated and set by an actual CE machine, may be set by the user, may be set by the user for each measurement, or may be set on a computer.

[0095]

<Noise of CE and Pull-up>

There is also an artifact generated in the CE detection unit.

[0096]

A pull-up peak indicates that a peak derived from another dye is erroneously detected. The pull-up is remarkably strong when the CE shows saturation, but may be detected even

when the CE does not show saturation.

[0097]

If a large amount of DNA is brought into the CE unit in the previous measurement, a carryover may occur and a peak may be detected in a next measurement.

[0098]

In addition, air bubbles mixed in the CE unit, background noise of the detection unit, and the like may be reflected in the electropherogram.

[0099]

An analytical threshold (AT) may be provided during analysis to prevent noise peaks from being erroneously used in the analysis. The analytical threshold may be set to obtain a sufficient SN ratio by measuring the background noise, may be set by the user, may be set by a device for each experiment, or may be preset. In addition, a program for determining whether a peak exceeding the AT is derived from the amplification product, derived from an electrophoresis failure of CE, or derived from various kinds of noise of CE may be stored and executed.

[0100]

The AT may be set in two or more stages, namely, a first reference value and a second reference value. If a peak has a peak intensity larger than a first reference, the peak is determined to be a true peak, and a peak having an intensity equal to or larger than a second reference but equal to or smaller than the first reference may be set to be requested to be reviewed made by a user or an expert, or may be set to be determined to be a peak when other setting conditions are satisfied. In addition, the amplification efficiency may vary depending on the locus, and a light emission efficiency or a noise intensity may vary depending on the dye, and thus the AT may be set for each locus or each dye.

[0101]

<Countermeasures against Artifact>

A countermeasure method against the above artifact will be described. Regarding DNA not deteriorated and DNA not containing a PCR inhibitor, data such as an appropriate peak intensity (AT or more and OS or less), PHR, and IPA can be acquired by inputting DNA in an amount falling within an analysis range of the STR-CE into the PCR, and the appearance of Ski slope can be reduced. In addition, even in the presence of deteriorated, trace, or inhibitory substances, the amount of information to be obtained can be maximized if the number of cycles and the amount of inputs are appropriate. There is a parameter set of the number of PCR cycles and the amount of DNA, which can reduce the appearance of artifacts to the minimum while maximizing the amount of information to be obtained.

[0102]

<Evaluation Method of CE Analysis Results>

When CE analysis results are provided to the user, it may indicate to the user whether the obtained CE data is useful or not, or to what extent the CE data is useful.

[0103]

When the CE analysis results are presented to the user, it may indicate to the user whether the obtained CE data is a complete DNA test result (Full profile). The user may be advised as to whether the obtained CE data needs to be analyzed again.

[0104]

FIG. 2 illustrates an evaluation flow for determining whether the obtained DNA profile is a full profile, or for informing the user of the quality of the data. This chart is an example, and the order may be changed or a part of the order may be omitted, a step not illustrated here may be included, and a part or all of the steps may be performed simultaneously so that a plurality of flags are given. In addition, a flow as illustrated in FIG. 2 may be performed for each peak or each locus, and it may be determined to be a full profile when all the loci satisfy all the references. When it is not determined to be a full profile, information on only the loci that satisfy the references may be collectively provided to the

user. In addition, for each locus, which reference is not satisfied or which reference is satisfied may be output and provided to a table or the like.

[0105]

201. Among all the peak intensities, no peak intensity saturates (OSs) a CE detection system. When there is an OS peak, the peak, locus, or analysis result may be flagged as OS.

[0106]

202. All the loci have one or more peaks exceeding the AT. When there is a locus in which no peak is detected, the locus or analysis result may be flagged as drop out (DO).

[0107]

203. When there is only one peak exceeding the AT, the peak has an intensity more than twice that of the AT (excluding genes in which only one peak is originally detected, for example, a locus of sex chromosome). When the peak has no intensity more than twice that of the AT, the peak, locus, or analysis result is flagged as inconclusive homozygous (IH flag).

[0108]

204. The intensity of the peaks located at a position shifted by ± 1 base from the main peak does not exceed an IPAT set at a peak ratio of 1%, 5%, 10%, 20% or 40% with respect to the intensity of the main peak. When the peak intensity exceeds the IPAT, the peak, locus, or analysis result is flagged as having high IPA intensity (IPA- flag or IPA++ flag).

[0109]

205. All the detected peaks are present in the DNA strand length that can be assigned to the DNA type. If no peak is assigned to the DNA type and corresponds to the IPA, the peaks are recognized as an Off ladder (OL) peaks, and the peak, locus, or analysis result is flagged as OL. The peak appearing at a position of the stutter peak does not need to be a determination target of the OL.

[0110]

206. Three or more peaks (two or more peaks for a locus that should only have one)

are not detected for one locus. If the peaks are detected, an intensity of a peak having a third highest intensity is 1% or less, 5% or less, 10% or less, 20% or less, or 40% or less with respect to an intensity of a peak having a second highest intensity. When a ratio of the intensity of the peak having the third highest intensity to the intensity of the peak having the second highest intensity exceeds a threshold, the DNA contained in the sample may be determined to be derived from two or more persons, and may be flagged as Mix. However, if the peak having the third highest intensity occurs at a position at which a first or second stutter peak appears, the peak having the third highest intensity may be determined to be a stutter peak. If a peak appears at a stutter peak position with an intensity of 1% or less, 5% or less, 10% or less, 15% or less, 20% or less, or 40% or less than that of the main peak, the peak may be determined to be a stutter peak and may not be flagged as Mix. If there is a peak at a position that may be a stutter peak and an intensity thereof exceeds a threshold of an allowable range for the stutter peaks, the peak may be a Mix, and thus the peak may be flagged as Mix. In addition, when the intensity of the peak having the third highest intensity at a position at which the stutter peak should not appear exceeds the threshold, the peak may be flagged as Mix. Similar determinations may be made for peaks having fourth, fifth, and subsequent intensities.

[0111]

207. When two or more peaks are detected, and the intensity of the peak having the second highest intensity is 1% or more, 5% or more, or 10% or more, and 60% or less, 40% or less, or 20% or less with respect to the intensity of the peak having the first highest intensity, the PHR may be determined to be poor, and the peak may be flagged as the PHR.

[0112]

The PHR becomes poor when the amount of DNA transferred to the PCR falls below 0.6 ng, 0.3 ng, 0.15 ng, or 0.075 ng. For example, when the approximate amount of DNA is estimated based on the intensity of the IPC or the number of PCR cycles and the intensity of

the peak in the CE analysis, if the PHR does not exceed the threshold although the amount of DNA transferred to the PCR exceeds the amount of DNA, it may be determined that the obtained DNA profile is derived from the mixed sample.

[0113]

A peak flagged as the IPA may also be flagged as the OL, and thus a determination reference 205 may also serve as 204, that is, the IPA flag may include the OL flag.

[0114]

When none of the flags are given, the full profile is obtained. If no full profile is obtained, a review made by the experts may be requested. In this case, it may take a long time for the DNA test to be completed. Therefore, it is necessary to increase a probability of achieving the full profile by setting an appropriate number of cycles. In addition, even in a case in which the samples are originally mixed, in a case in which the amount is small, in a case in which there are a lot of PCR inhibitors, in a case in which no full profile is obtained but all of the loci are not normally detected, and the like, when 5 or more or 10 or more loci are detected, the loci can be used for criminal investigation. Such a DNA profile is called a partial profile. The more information on the obtained loci, the more useful the information is in the criminal investigation. That is, even in a situation in which the partial profile is obtained, it is necessary to set the analysis protocol, particularly the number of PCR cycles, such that as many peaks as possible can be detected. In particular, in the case of the mixed sample or in a case in which the amount of DNA is small, it is necessary to set a protocol, in particular, the number of PCR cycles such that as many peaks as possible can be detected while the electropherogram satisfies 201, 205, and 206 (that is, the flags are not given).

[0115]

The threshold may be set such that it can be confirmed whether the DO is caused because the DNA input to the PCR reaction does not contain the allele or because the number of PCR cycles is small. For example, when there is a locus that has no peak exceeding the

AT, and when there is a peak that is 10 times larger than the AT in the entire amplification products, it may be determined that the DO is due to the sample, otherwise, it may be determined whether the DO is due to a low number of PCR cycles or the DO is due to the sample. This determination may be a material for determining whether to perform analysis with an increased number of PCR cycles.

[0116]

[Regarding Analysis Range]

When the STR-CE is performed, factors that cause the intensity to fluctuate are as follows: (1) fluctuation in the concentration of salts or other injection-inhibited substances contained in the PCR reaction solution, (2) a mixing ratio of the electrophoresis reagent and the PCR reaction solution, (3) the amplification efficiency, (4) the deterioration of the electrophoresis reagent or incomplete denature, (5) a variation in temperature of the electrophoresis unit, (6) a variation during electrolytic injection, (7) a variations between capillaries and between capillary arrays, and (8) a variation in a detected intensity of the detection unit.

[0117]

Hereinafter, the dynamic range of the CE may be a dynamic range described in a manual or the like set in various CE devices, a ratio of a maximum amount of amplicons to a minimum amount of amplicons at which obtain a signal is linearly obtained with respect to the amount of amplicons actually input to the CE, the maximum amount of amplicons to the minimum amount of amplicons to which a relative ratio of the amount of amplicons input to the CE can be assigned based on a signal intensity, and may be a ratio of any upper limit of analysis to any lower limit of analysis. The dynamic range of the CE may be a ratio of the OT to the AT. The dynamic range of the CE may vary for each measurement.

[0118]

Hereinafter, the analysis range of the STR-CE may simply correspond to the dynamic

range of the CE unless otherwise specified, the amount of DNA capable of correctly detecting a specific allele may be examined by performing an experiment while actually changing the amount of DNA put into the PCR in the experiment, the amount of DNA capable of correctly detecting a specific allele set may be examined by performing an experiment while actually changing the amount of DNA put into the PCR in the experiment, and a range of the amount of DNA may be experimentally examined for a plurality of different persons, and the average, minimum, or maximum range may be set as the analysis range. The amount of DNA used for the CE measurement is generally proportional to the amount of DNA put in the STR-PCR, but is not necessarily proportional to the amount of DNA because of the DNA concentration dependence of the CE injection efficiency, the DNA concentration dependence of the PCR amplification efficiency, and the cycle number dependence. In addition, the analysis range of the STR-CE does not change significantly in most cases when the number of PCR cycles is changed, but in particular, when the amount of DNA is reduced, the analysis range may be shifted due to a stochastic effect (stochastic effect in which the amount of DNA corresponding to a locus to be amplified is not proportional to the amount of DNA input). Therefore, it is desirable to perform the above examination for each cycle to determine a true analysis range of the STR-CE, and the analysis range for each cycle set through such examination is also considered to be a type of analysis range of the STR-CE. A large amount of labor is required to confirm all the analysis range of the STR-CE in the experiment, and thus a part thereof may be replaced by calculation.

[0119]

The dynamic range of the CE or the analysis range of the STR-CE may be evaluated taking into consideration of the variations (1) to (8) described above.

[0120]

By changing the set threshold, the analysis range can be widened or narrowed. When the analysis range is widened, there is a possibility that a compensation in which the

accuracy decreases occurs. When an artifact and a true peak can be more reliably separated by using a method such as machine learning, the threshold can be changed in a direction in which the analysis range is widened.

[0121]

Hereinafter, the analysis range of the analysis system refers to, for example, a range of the amount of DNA contained in the sample in which the DNA analysis can be correctly performed on the DNA sample input to the analysis system. In order to widen the analysis range, it is necessary to widen the analysis range of the STR-CE or control the amount of DNA input to the STR-CE. When the analysis range of the STR-CE is widened, it is possible to improve the dynamic range and the sensitivity of the CE, to reduce the variation of the STR-CE, to prepare a plurality of cycles of amplification products, or to prepare eluates at different dilution rates and subject the eluents to the STR-CE. When the amount of DNA input to the STR-CE is controlled, a volume of a purified film may be reduced or meshes may be made rough so that the upper limit of the amount of DNA that can be treated by the purified film can be cut off, or the dilution rate may be changed by quantifying the amount of DNA after purification.

[0122]

<<Analysis System for Split PCR and Operation Example thereof>>

[0123]

[Analysis System]

[Configuration Example of General Analysis System]

FIG. 3 illustrates an example of the analysis system 101. FIG. 4 illustrates an example of an operation procedure of the analysis system 101. A biomolecule analyzer includes the computer 102 for performing biomolecule analysis and the flow path device 104.

[0124]

The flow path device 104 includes a dissolution chamber 301 for introducing and

dissolving a collected sample, a purified film chamber 303 in which a purified film 302 is stored, a PCR chamber 304 (PCR chamber for performing a thermal cycle) for performing DNA amplification and the like, and a waste liquid chamber 305. The flow path device 104 further includes an external connection port 306 fluidly connected to the outside of the device. The solution is transported via the external connection port 306, and a reagent, an amplification product, and the like can be exchanged with the outside of the device. When a solution is transported in the analysis system 101, the transport of the solution may be controlled by using a pump and valve 307. The pump and valve 307 may be provided entirely outside the flow path device, or a part of the pump and the valve 307 may be provided within the flow path device 104. A PCR reagent storage unit 308 may include a PCR reagent 309 (polymerase, Primer, dNTP, buffer, and the like) necessary for the PCR reaction, and an electrophoresis reagent storage unit 310 may include an electrophoresis reagent 311. In addition, reagent storage units 312, 313, and 314 for storing reagents necessary for pretreatment are also included. In a sample input step 401, before and after the sample is stored in the dissolution chamber 301, a dissolution buffer is transported from the reagent storage unit 312 to the dissolution chamber 301. Next, the dissolution starts in the dissolution step 402. In a purification step 403, a dissolution product is sent from the dissolution chamber 301 to the purified film chamber 303, DNA is bound to the purified film 302, and a washing liquid is released from the reagent storage unit 313 to perform purification. After the purification, a step of drying the washing liquid or the like may be included. The eluate is released from the reagent storage unit 314, and the DNA eluted from the purified film chamber 303 is transported to the PCR chamber 304. The PCR reagent is transported from the PCR reagent storage unit 308 to the PCR chamber 304 and mixed with the eluted DNA. In an amplification step 404, the DNA purified in the PCR chamber 304 is mixed with the PCR reagent 309 and subjected to the PCR reaction. The DNA amplified in a detection step 405 is mixed with the electrophoresis reagent 311 stored in the electrophoresis reagent storage

unit 310, and measured by the CE unit 105.

[0125]

After the electrophoresis reagent and the PCR reaction solution are mixed with each other, a step of heating the same at 80°C to 100°C and rapidly cooling the same to 0°C to 10°C may be provided before the CE analysis. By providing this step, DNA is more completely single-stranded, and highly accurate CE analysis is implemented.

[0126]

[Example of Analysis System used in Present Embodiment]

FIG. 5 illustrates an outline ((1) of FIG. 5) and an example ((2) of FIG. 5) of an operation procedure of the analysis system 101 of the present embodiment. Operation steps 501 to 507 may correspond to the amplification step 404 and the detection step 405.

[0127]

As illustrated in (1) of FIG. 5, the analysis system 101 of the present embodiment includes the flow path device 104 and the CE unit 105, and the flow path device includes the PCR chamber 304. In addition, the analysis system 101 includes a dispensing chamber 320. The PCR reaction solution or the electrophoresis sample is fed into the dispensing chamber 320. The dispensing chamber 320 may be omitted. The respective elements are connected by flow paths 315 and 316.

[0128]

As illustrated in (2) of FIG. 5, the analysis system 101 prepares the PCR reaction solution in step 501, performs m PCR cycles in the PCR chamber 304 in step 502, takes out the PCR reaction solution m from the PCR chamber 304 without changing compositions of a part of the amplification product in step 503, subjects the electrophoresis sample m to the CE measurement by the CE unit 105 in step 504, and subjects the PCR reaction solution m remaining in the PCR chamber 304 to n - m thermal cycles in step 505, thereby obtaining the PCR reaction solution n. In step 506, a part of the PCR reaction solution n is taken out from

the PCR chamber 304 without changing the compositions, and in step 507, the electrophoresis sample n is subjected to the CE measurement by the CE unit 105.

[0129]

A dispensing step in step 506 may be omitted, and all the amplification products in n cycles may be mixed with the electrophoresis reagent in step 507.

[0130]

The division number is increased to three, four, or more by repeating steps 503 to 505 a plurality of times.

[0131]

Steps 504 and 507 may be performed simultaneously, or step 507 may be performed after step 504. By performing step 507 after step 504, the settings of step 505 can be changed depending on the result of step 504.

[0132]

After step 503 ends, step 505 may start immediately. A time between step 502 and step 505 is short, and thus the occurrence of artifacts can be reduced. In addition, execution timings of step 505 and step 506 may be set such that step 507 can start at an end timing of step 504.

[0133]

[Derived Example of Analysis System Used for Implementation of Present Invention]

As illustrated in FIG. 6, the analysis system 101 may include heating units 317 and 318 (temperature control and heating mechanism) for performing a thermal cycle in the PCR chamber 304. A flow path 319 and the flow path 315 are connected to the PCR chamber 304, and the PCR chamber 304 may receive the supply of a reagent or control of a pressure. As illustrated in FIG. 6, the analysis system 101 may include a flow path device, and the pump and valve 307 for transporting a solution suitable for various steps such as the CE measurement. As illustrated in FIG. 6, the analysis system 101 may include the computer

102, and the computer may have a function of controlling the pump and valve 307, a function of controlling the CE measurement, a function of controlling a temperature, a function of analyzing and feeding back data obtained from the CE unit 105, the heating units 317 and 318, and the like, a function of providing the data to the user, and the like. The flow path device may include the dispensing chamber 320. The dispensing chamber 320 has a function of taking out a part of the amplification product from the PCR chamber 304 without changing the compositions at a timing when m thermal cycles are completed. The dispensing chamber 320 may have a metering function of taking out a specified amount of the PCR reaction solution m or n. When the PCR reaction solution is fed into the dispensing chamber 320, the dispensing chamber 320 may aspirate the PCR reaction solution, or the PCR reaction solution may be fed into the dispensing chamber 320 by a pressure. In addition, the dispensing chamber 320 may be provided only for temporarily storing the PCR reaction solution, and may not have the metering function. The dispensing chamber 320 may be provided outside the flow path device 104.

[0134]

The PCR reaction solution m may be allowed to wait in the dispensing chamber 320 for a period of time until the CE measurement is performed. The PCR reaction solution m may be mixed with the electrophoresis reagent (formamide or size standard) in the dispensing chamber 320. That is, the analysis system 101 mixes the reaction solution (for example, at least one of the part of the PCR reaction solution m and the at least part of the PCR reaction solution) with a solution containing a plurality of types of DNA fragments. Accordingly, the concentration can be determined based on a peak height of the size standard.

[0135]

As illustrated in FIG. 6, the analysis system 101 may include a standby unit 321 between the CE unit 105 and the PCR unit. The standby unit 321 may temporarily hold the PCR reaction solution or the electrophoresis sample in which the PCR reaction solution and

the formamide are mixed in steps 506 to 507 or steps 503 to 504. The flow path device (and the following drawings) of FIG. 6 may be disposed in a longitudinal direction, that is, may be disposed such that a main portion of the flow path or at least a part of the flow path is parallel to the gravity direction. In one form of the flow path device, a lower side in the drawing is used downward in the gravity direction. Air bubbles and mixed air in each chamber are accumulated in an upper portion of the chamber in the vertical direction, and thus it is possible to minimize the mixing of air into a next step by taking out the solution from a lower portion of the chamber when transporting to a next chamber or a next flow path.

[0136]

The flow path device can be modified as follows.

[0137]

As illustrated in FIG. 6, in one form of the analysis system 101 of the present embodiment, the flow path device includes the CE unit 105. In addition, as illustrated in FIG. 7, in one form of the analysis system 101, the flow path device includes the pump and valve 307. An optical system may be disposed outside the flow path device or inside the analysis system 101.

[0138]

[Flow Path Device]

As illustrated in FIG. 8, the flow path device 104 may receive the taking-out of the solution and the control of air pressure via the analysis system 101 and the external connection port 306. A pressure may be applied from the external connection port 306 via a flow path 322, and the PCR product or the electrophoresis sample may be pumped out from the dispensing chamber 320. The flow path device includes valves 323, 324, 325, and 326, which are opened and closed depending on the analysis step.

[0139]

As illustrated in FIG. 9, the flow path device may include a mixing chamber 327

between the dispensing chamber 320 and the external connection port 306. The PCR reaction solution in the dispensing chamber 320 and the electrophoresis reagent may be mixed in the mixing chamber 327. The mixing chamber 327 may be connected to the dispensing chamber 320 by a flow path 328. The mixing chamber 327 may be provided outside the flow path device and inside the analysis system 101, and in some cases, the standby unit 321 may take on this role. A flow path 329 is connected to the mixing chamber 327, and by applying a pressure to the flow path 329, air bubbles may be fed into the mixing chamber 327 for mixing, or an electrophoresis sample may be fed into the CE unit 105.

[0140]

As illustrated in FIG. 9, the PCR chamber 304 may include three flow paths 319, 315, and 330. The flow path 319 may be connected upstream of the sample, the flow path 315 may be connected to the dispensing chamber 320, and the flow path 330 may be connected to the mixing chamber 327. The PCR reaction solution m taken out from the PCR chamber 304 may be transported to the mixing chamber 327 via the dispensing chamber 320, and the PCR reaction solution n taken out from the PCR chamber 304 may be transported to the mixing chamber 327 via the flow path 330 without passing through the dispensing chamber 320. The PCR reaction solution m and the PCR reaction solution n pass through different paths, and thus a decrease in reproducibility caused by the remaining solution can be prevented. In addition, when the reaction solution n is metered, and the entire amount of the reaction solution m is transported to the mixing chamber 327 or the CE unit 105 without being metered, it is not necessary to transport the reaction solution m to the dispensing chamber 320, and thus the structure of the present flow path device can perform split PCR more easily.

[0141]

When the PCR reaction solution m is also metered, another dispensing chamber may be provided separately from the dispensing chamber 320 used for the PCR reaction solution n.

[0142]

As illustrated in FIG. 10, the flow path device 104 may include electrophoresis reagent storage units 310 and 331 for storing electrophoresis reagents (such as formamide, DNA fragments, and pure water). The electrophoresis reagent storage units 310 and 331 may be located on the flow path 315 or on the flow path 319. The reagent storage unit may be divided into two or more storage units for one type of reagent as illustrated in FIG. 10. By dividing the reagent storage unit into two storage units, a specified amount of reagent can be released from each of the storage units at the time of division. Alternatively, the amount of reagent released from the storage unit may be controlled, and the reagent may be released from one reagent storage unit two or more times. As illustrated in FIG. 10, the flow path device 104 may include air storage units 332 and 333. The air storage units 332 and 333 may be located on the flow path 315 or on the flow path 319. At the time of division, a specified amount of PCR reaction solution can be transported to the outside of the PCR chamber 304 by releasing a specified amount of air from the air storage unit 332 or 333. In the case of the configuration of the flow path device, the split PCR may also be performed when the dispensing chamber 320 is omitted. In the case of the configuration of the flow path device, the mixing chamber 327 may also serve as the dispensing chamber 320. The air storage unit 332 or the air storage unit 333 may contain a liquid that does not affect the PCR reaction, such as oil, instead of air. The air storage unit 332 or 333 may contain the PCR reaction solution, and a PCR reaction solution having a volume corresponding to the pushed-out PCR solution may be newly replenished.

[0143]

As illustrated in FIG. 11, the electrophoresis reagent storage units 310 and 331 may be provided in a pair of flow paths across the PCR chamber 304. As illustrated in FIG. 11, the electrophoresis reagent storage unit 331 is preferably disposed closer to the PCR chamber 304 than the air storage unit 333. This is because all or more of the electrophoresis reagent

311 accumulated in the flow path 319 or the PCR chamber 304 can be fed into the dispensing chamber 320 or the mixing chamber 327, thereby contributing to improvement in the reproducibility. As illustrated in FIG. 11, the flow path device 104 may include an air storage unit 334 closer to the PCR chamber 304 than the electrophoresis reagent storage unit 310 on the flow path 315. The air storage unit 334 may be used to transport the electrophoresis sample m in the dispensing chamber 320 to the mixing chamber 327 or the CE unit 105. The air storage unit 334 may be replaced with the flow path 322.

[0144]

[Example of Implementation Method of Split PCR]

In this section, a method for performing split PCR on the flow path device 104 will be described. Although a dispensing chamber is mentioned throughout, solutions may be transported directly to the CE unit 105 without using the dispensing chamber or the mixing chamber.

[0145]

FIG. 12 illustrates an example of the flow path device 104, and FIG. 13 illustrates an example of a transport method for performing the split PCR on the flow path device of FIG. 12. For convenience of illustration, a part of the reference numerals are illustrated in FIG. 12 but omitted in FIG. 13.

[0146]

Step I: there is the PCR chamber 304 containing a PCR reaction solution 335, and m cycles of PCR reaction are performed in the PCR chamber 304 with valves 326, 323, and 325 closed. This step may correspond to step 502.

[0147]

In this way, the analysis system 101 generates the PCR reaction solution m (first reaction solution) by subjecting the PCR reaction solution 335 to m thermal cycles in the PCR chamber 304.

[0148]

Step II: after m cycles of the PCR reaction are completed, the valves 326 and 323 are opened, and a part of the solution in the PCR chamber 304 is transported to the dispensing chamber 320. This step may correspond to step 503.

[0149]

In this way, the analysis system 101 takes out the part of the PCR reaction solution m from the PCR chamber 304 without changing compositions thereof. Here, the analysis system 101 may subject the part of the PCR reaction solution m to the electrophoresis analysis in the CE unit 105.

[0150]

In this way, the flow path device 104 includes the openable and closable valves 326 and 323, and the part of the PCR reaction solution m is divided and taken out by closing the valves 326 and 323 before the start of the m thermal cycles and opening the valves 326 and 323 after the completion of the m thermal cycles. In this way, a division process can be performed appropriately.

[0151]

Step III: the electrophoresis reagent 311 is pushed out from the electrophoresis reagent storage unit 310 by closing the valves 323 and 326, and the PCR reaction solution m stored in dispensing chamber 320 is transported to the mixing chamber 327. The PCR reaction solution m becomes an electrophoresis sample 336 by being mixed with the electrophoresis reagent 311.

[0152]

Step IV: a valve 325 is opened, and the electrophoresis sample in the mixing chamber 327 is transported to the outside of the flow path device 104 (standby unit 321 or CE unit 105 of the analysis system). This step may correspond to step 504.

[0153]

Step V: the valve 326 is closed, and $n - m$ thermal cycles are executed. This step may correspond to step 505.

[0154]

In this way, the analysis system 101 generates the PCR reaction solution n (second reaction solution) in the PCR chamber 304 by subjecting the PCR reaction solution m remaining in the PCR chamber 304 to $n - m$ (where $n - m$ is an integer of 2 or more) thermal cycles such that the total number of thermal cycles is n .

[0155]

Step VI: after a total of n thermal cycles are completed, the valve 325 is closed, the valves 326 and 323 are opened, and the PCR reaction solution n is transported to the mixing chamber 327.

[0156]

In this way, the analysis system 101 takes out the at least part of the PCR reaction solution n from the PCR chamber 304 without changing the compositions thereof.

[0157]

The electrophoresis reagent is pushed out from the electrophoresis reagent storage unit 331, and the PCR reaction solution n stored in the dispensing chamber 320 is transported to the mixing chamber 327. The valve 325 is opened, and the electrophoresis sample in the mixing chamber is transported to the outside of the flow path device 104 (standby unit 321 or CE unit 105 of the analysis system). This step may correspond to step 507.

[0158]

The analysis system 101 may subject the at least part of the PCR reaction solution n to the electrophoresis analysis in the CE unit 105.

[0159]

[Other Examples of Implementation Method of Split PCR]

FIG. 14 illustrates an analysis process of the split PCR, FIG. 15 illustrates an example

of the flow path device 104, and FIG. 16 illustrates an example of a transport method of dividing the PCR reaction solution into $m = 24$ cycles and $n = 30$ cycles in FIG. 15. For convenience of illustration, a part of the reference numerals are illustrated in FIG. 15 but omitted in FIG. 16.

[0160]

The flow path device 104 includes a valve 337 on the flow path 315 that connects the dispensing chamber 320 and the PCR chamber 304. The valve 337 is disposed closer to the PCR chamber 304 than a branch to the electrophoresis reagent storage unit 310. In addition, the flow path 316 is provided with a valve 338. In addition, the flow path 319 is provided with a valve 339 closer to the flow path 329 than a branch to the electrophoresis reagent storage unit 331.

[0161]

Step I: all the valves are closed. There is the PCR chamber 304 containing a μl of the PCR reaction solution 335, and 24 thermal cycles are performed in the PCR chamber 304. After 24 thermal cycles are completed, the final extension is performed for 8 minutes (steps 601 to 604 may correspond to steps 501 and 502).

[0162]

Step II: after 24 cycles of the PCR reaction are completed, the valves 326, 323, and 337 are opened, and a part ($b \mu\text{l}$) (where $a > b$) of the solution in the PCR chamber 304 is transported to the dispensing chamber 320 via the flow path 315 (this step may correspond to step 605 and step 503).

[0163]

Step III: $c \mu\text{l}$ of the electrophoresis reagent 311 is pushed out from the electrophoresis reagent storage unit 310 by closing the valves 323, 326, and 337 and opening the valves 324 and 325, and the PCR reaction solution m stored in dispensing chamber 320 is transported to the mixing chamber 327. The PCR reaction solution m becomes the electrophoresis sample

336 by being mixed with the electrophoresis reagent 311 (this step may correspond to step 606 and step 504). At this time, the electrophoresis reagent 311 may remain in the dispensing chamber 320 and a part of the flow path 315.

[0164]

Step IV: the electrophoresis sample in the mixing chamber 327 is transported to the outside of the flow path device 104 (standby unit 321 or CE unit 105 of the analysis system) by closing the valve 324 and opening the valve 338 (this step may correspond to step 607 and step 504).

[0165]

Step V: all the valves are closed, and a - b μ l of the PCR reaction solution 335 remaining in the PCR chamber 304 is subjected to 6 thermal cycles, and then the final extension is performed for 8 minutes (this step may correspond to steps 608 to 610 and step 505).

[0166]

Step VI: after a total of 30 thermal cycles are completed, the valves 326, 323, and 325 are opened, d μ l of the electrophoresis reagent is pushed out from the electrophoresis reagent storage unit 331, and the PCR reaction solution n is transported to the mixing chamber 327 (this step may correspond to step 506).

[0167]

Step VII: the valve 325 may be closed, the valve 339 may be opened, air pressure may be applied, and all the solution remaining in the PCR chamber 304 may be fed into the mixing chamber 327. In addition, the electrophoresis sample 336 may be stirred and homogenized by feeding air into the mixing chamber 327 (this step may correspond to step 611 and step 507).

[0168]

Step VIII: the valve 338 is opened, and the electrophoresis sample in the mixing

chamber 327 is transported to the outside of the flow path device 104 (standby unit 321 or CE unit 105 of the analysis system) (this step may correspond to step 612 and step 507).

[0169]

Table 1 shows an example of a time and a temperature in each step of the split PCR process illustrated in FIG. 14.

[0170]

[Table 1]

Table 1 Split PCR Protocol

Step	Details of steps	Time	Temperature	Number of cycles
603	Initial Denaturation	60 s	95°C	24 cycles
	Denaturation	3 s	94°C	
	Annealing & Extension	30 s	60°C	
604	Final Extension	480 s	60°C	
605	Dispensation		RT~95°C	
608	Denaturation	3 s	94°C	6 cycles
	Annealing & Extension	30 s	60°C	
609	Final Extension	480 s	60°C	

[0171]

The setting of a basic thermal cycle was performed with reference to a manual (https://assets.thermofisher.com/TFS-Assets/LSG/manuals/4477672_GlobalFilerExpress_UG.pdf) of the STR-PCR kit (GlobalFiler (trademark) Express). In step 605, the temperature of the PCR chamber 304 during dispensing may be set to any temperature between the room temperature and Denaturation.

[0172]

In steps 605, 606, 610, and 611, the solution may be maintained at a low temperature (4°C). By maintaining the solution at a low temperature, it is possible to prevent the

deterioration of the sample or the progress of an unnecessary reaction.

[0173]

In step 605, it is preferred to set the temperature at the time of division to be substantially the same as a temperature in the Denaturation step, so that an unnecessary extension reaction can be prevented, and the occurrence of artifacts can be reduced.

[0174]

In step 605, when the temperature at the time of division is set to be equal to the extension temperature, the unnecessary extension reaction occurs, but non-specific amplification can be reduced. In addition, this is preferable because it is possible to avoid the inactivation of the polymerase or the fluorophore. The influence of the unnecessary extension reaction on the analysis accuracy of the STR-CE can be ignored.

[0175]

In step 605, the temperature at the time of division may be set between the extension temperature and the RT. In this case, accurate temperature control is not required, which is convenient. At the completion of m cycles of the PCR, sufficient amplicons are present, and thus the influence of the non-specific amplification can be ignored.

[0176]

In step 605, the shorter the time required for division, the more preferable. If the time required for division is long, the amount of artifacts may increase. In addition, as the time required for division is longer, there is a possibility that the inactivation of various biomolecules such as polymerases proceeds.

[0177]

As illustrated in FIG. 14, in the present embodiment, the analysis system 101 does not subject each reaction solution (including the part of the PCR reaction solution m and the at least part of the PCR reaction solution n) to analysis using a method other than the electrophoresis analysis before the electrophoresis analysis. In this way, the configuration

of the device becomes simple and, for example, an additional optical system becomes unnecessary.

[0178]

[Amount of PCR Reaction Solution and Electrophoresis Reagent]

The amount of the PCR reaction solution used in the flow path device is 1 μl to 200 μl , more preferably 10 μl to 50 μl . As the amount of solution is smaller, the more accurate and high-speed temperature control can be performed. On the other hand, as the amount of solution is larger, a larger amount of purified DNA can be received, and thus higher sensitivity is likely to be achieved. In addition, as the amount of solution is larger, highly accurate metering of solution at the time of division becomes unnecessary.

[0179]

Table 2 shows an example of the amount of solution at the time of division. An amount of solution actually metered does not have to be a value in the table, and a median value obtained by measuring the amount of solution a plurality of times may be a value in the table.

[0180]

[Table 2]

Table 2 liquid Amount for Split PCR

	a [μl]	b [μl]	c [μl]	d [μl]
Set 1	a	b	10a	10(b - a)
Set 2	15	5	50	100
Set 3	a	b	10a + e	10(b - a)
Set 4	a	b	11a	10(b - a)

[0181]

It is assumed that the total amount of the PCR reaction solution is a μl in step 502, the amount of solution taken out m cycles in step 503 is b μl , the electrophoresis reagent mixed

with the PCR reaction solution m in step 504 is c μ l, and the electrophoresis reagent mixed with the PCR reaction solution n in step 507 is d μ l. Table 2 shows preferred examples of relation expressions a to d or set values. In order to efficiently introduce an amplification product or a size standard into the CE, an ion intensity of the electrophoresis sample needs to be sufficiently low. Therefore, it is preferred that the amount of the electrophoresis reagent is mixed larger than that of the PCR reaction solution at a ratio of 2 times, 5 times, 10 times, 20 times, and the like in volume with respect to the amount of the PCR reaction solution.

[0182]

The split PCR may be set such that the PCR reaction solution m is equal to the PCR reaction solution n. When the amounts of the PCR reaction solutions m and n are close to each other, the stable transport of the electrophoresis reagent to the CE unit can be implemented.

[0183]

The split PCR may be set such that the PCR reaction solution m is smaller than the PCR reaction solution n. The smaller the amount of solution taken out at the time of division, the smaller the variation in the amount of solution when n - m PCRs are performed, and thus the amplification product n can be prepared with higher reproducibility.

[0184]

Set 1 is an example of an amount of solution when the amount of the electrophoresis reagent is set to 10 times the amount of PCR reaction solution.

[0185]

Set 2 is an example of an amount of solution when an amount of PCR reagent is 15 μ l, 5 μ l of the solution is taken out at the time of division m cycles, and the entire amount of PCR reaction solution is mixed with the electrophoresis reagent during n cycles. In this example, the DNA analysis system 101 transports an entire amount of the PCR reaction solution in the PCR chamber to the outside of the PCR chamber at the completion of n thermal

cycles. In this way, a device necessary for metering can be omitted, and the configuration of the flow path is simplified.

[0186]

In the solution transport procedure illustrated in FIG. 16, the electrophoresis reagent remains in the dispensing chamber 320. When the amount of electrophoresis reagent in the electrophoresis reagent storage unit 310 is sufficiently large with respect to the dispensing chamber 320, the influence on the analysis accuracy or the analysis range can be ignored even if the electrophoresis reagent remains in the dispensing chamber 320. In addition, if the flow path 322 is provided as an air line between the valve 337 and the dispensing chamber 320, or the air storage unit 334 is provided on the flow path 315, the entire amount can be pushed to the dispensing chamber 320 and the mixing chamber 327. When the volume is large, almost the entire amount of the PCR reaction solution m in the dispensing chamber 320 is fed into the mixing chamber. In addition, taking into consideration that a part of the electrophoresis reagent remains in the dispensing chamber 320, it is preferred to store an excess of the electrophoresis reagent in the reagent storage unit.

[0187]

Set 3 is an example of an amount of solution when a large amount of the electrophoresis reagent is stored in the electrophoresis reagent storage unit 310 taking into consideration that $e \mu\text{l}$ of the electrophoresis reagent remains in the dispensing chamber 320.

[0188]

Set 4 is an example of an amount of solution when a large amount of the electrophoresis reagent is stored in the electrophoresis reagent storage unit 310 taking into consideration that a volume of the dispensing chamber 320 is $b \mu\text{l}$, and $b \mu\text{l}$ of the electrophoresis reagent remains in the dispensing chamber 320.

[0189]

[Detailed Division Method]

The variation in the amount of solution at the time of division acts in a direction to narrow an effective analysis range of the analysis system, and thus it is desirable that the accuracy of solution division is high when the PCR reaction solution is divided. In order to implement highly accurate solution division, a function such as setting an appropriate pressurizing pressure and pressurizing time in advance, pushing out the PCR reaction solution with a liquid or gas having a specified volume, and pushing out the PCR reaction solution into the dispensing chamber 320 with a specified volume using a liquid surface detection sensor may be incorporated. A plurality of pushing-out methods and metering methods may be combined.

[0190]

The metering may be performed when the PCR reaction solution m is dispensed in step 503 and when the PCR reaction solution n is dispensed in step 506. When step 506 is omitted and the PCR reaction solution n is mixed with the entire amount of the electrophoresis reagent, the metering may be performed only in step 503. That is, the number of times of metering may be a number obtained by subtracting 1 from the division number. Similarly, when the metering is performed twice, there is a specified amount of solution in the PCR chamber, and thus the reaction solution remaining in the PCR chamber is additionally subjected to p thermal cycles of PCR, and the reaction solution in a third division subjected to $n + p$ cycles of the PCR reaction may be subjected to the CE measurement.

[0191]

When the PCR reaction solution is taken out from the PCR chamber, the PCR chamber may be pressurized by a pump to be pumped to the dispensing chamber. In addition, when the valve is closed at the time of heating the PCR, an internal pressure is applied, and thus the solution may move to the dispensing chamber by the internal pressure without opening the valves 326 and 323 in step II.

[0192]

After m cycles are completed in step 502, the PCR reaction solution m may be pushed out with a new PCR reagent in step 503, and a specified amount may be transported to the dispensing chamber 320 or the mixing chamber 327. This division method may be performed by sealing the air storage unit 332 in FIG. 10 with the PCR reagent instead of air. When the PCR reaction solution m is pushed out with a solution, there is an advantage that a volume to be pushed out is likely to be defined. In addition, in the case of being pushed out with the PCR reaction solution, there is an advantage that there is no bad influence on the reaction due to the inclusion of air bubbles or oil. On the other hand, there is a possibility that a new PCR reagent goes to subsequent stages, and a new factor is added in addition to the above factors (1) to (8) that cause the variation of the analysis range. In addition, the PCR reaction solution m is diluted with the PCR reaction solution, and thus the PCR reaction solution remaining in the PCR chamber is diluted at the end of m cycles, and more PCR cycles are required. Further, when a room-temperature reagent is added, the temperature of the PCR chamber decreases. Furthermore, it is necessary to perform the initial denature again in step 505. Therefore, in the case of being pushed out with the PCR reaction solution, a PCR reaction time becomes slightly longer. Furthermore, increasing the number of cycles and performing the initial denature again contribute to the occurrence of artifacts. It is also possible to solve a part of the above problems by performing the initial denature of the PCR reaction solution used for pushing out before step 503.

[0193]

As illustrated in FIG. 10, the air storage units 332 and 333 may be disposed in the flow path device 104, the PCR reagent may be pushed out in the air chamber at a timing when the m cycles are completed in step 502, and a specified amount may be transported to the dispensing chamber 320 or the mixing chamber 327 in step A3.

[0194]

The PCR reaction solution may be pushed out with oil instead of air or the PCR

reagent. This division method may be performed by sealing the air storage unit 332 in FIG. 10 with oil instead of air. The oil may be any oil that does not affect the PCR reaction. For example, the oil may be silicone oil, mineral oil, fluorinert oil, or a mixture of a plurality of oils. In the case of being pushed out with the oil, the volume to be pushed out is likely to be defined as in the case of being pushed out with the reagent, and unlike the case of being pushed out with the PCR reaction solution, there is an advantage of not being mixed with the PCR reaction solution. On the other hand, when the oil is mixed in the PCR reaction product and flows to the subsequent stages, the CE measurement may be hindered. When the PCR reaction solution m is taken out, the PCR reaction solution m may be separated by a separation membrane or by centrifugation for leaving the oil in the chamber.

[0195]

[Details of Metering Mechanism]

When the PCR reaction solution is introduced into the dispensing chamber 320 at the time of division, if air or air bubbles enter the PCR chamber instead of the PCR reaction solution, the metering accuracy decreases. Therefore, it is preferred that the dispensing chamber 320 and the flow path 315 are designed to prevent air from entering. FIG. 17 illustrates an example of a structure for preventing air from entering. As illustrated in (a) and (b) of FIG. 17, the flow path 315 branches off from the PCR chamber 304 below a liquid surface 701 of the PCR reaction solution 335 in the gravity direction. Air accumulates on an upper side of the PCR chamber 304, and thus the amount of air introduced into the flow path 315 at the time of division can be minimized. In addition, it is desirable to design the amount of solution or a chamber shape such that the liquid surface 701 is located above a connection portion between the PCR chamber 304 and the flow path 315 in the gravity direction when the division is completed. As illustrated in (b) of FIG. 17, the liquid surface 701 may be located above the valve 326 or 323 in the gravity direction. In this case, bubbles entering the PCR chamber 304 can be minimized or completely eliminated. The PCR reaction

solution 335 located above the valve 326 or 323 may not be subjected to the PCR reaction. In this case, the unreacted reagent may be mixed into the CE measurement, decreasing the sensitivity.

[0196]

As illustrated in (c) of FIG. 17, the flow path 315 may be connected to a bottom portion of the PCR chamber 304. In this configuration, there is an advantage that air is less likely to enter, but the flow path 315 is slightly long and tends to cause liquid loss.

[0197]

The flow path 315 may be provided with a structure for removing air bubbles. In addition, a hydrophilic filter that does not allow air to pass through a flow path that connects the PCR chamber 304 and the dispensing chamber 320 may be provided in the flow path 315.

[0198]

The dispensing chamber 320 may have the metering function. For example, FIG. 18 illustrates an example of the dispensing chamber 320 having the metering function. The flow paths 315 and 328 connected to the dispensing chamber 320 are provided with the valves 324 and 337. The valve 337 may be used not only for metering but also for preventing the liquid from jumping out into the dispensing chamber 320 during the PCR reaction or for preventing the electrophoresis reagent and the electrophoresis sample from flowing back to the PCR chamber 304 after being dispensed. In addition, the valve 324 may be provided not only for metering but also for preventing the electrophoresis sample from flowing back after being transported to the mixing chamber. The volume of the dispensing chamber may coincide with the amount of solution to be measured by dividing the amplification product m . The dispensing chamber may have a spherical shape or a cylindrical shape, may have a rectangular shape, may have an elongated shape, may have a meandering flow path, and may have an elliptical shape or an elliptical cylindrical shape.

[0199]

For example, the analysis system 101 may meter a predetermined amount of the PCR reaction solution m in a range of 0.1% to 50% by transporting the PCR reaction solution m to the dispensing chamber 320 (metering unit) after completion of the m thermal cycles. In this way, no additional metering process is required.

[0200]

FIG. 19 illustrates an example of the metering mechanism. A plurality of mechanisms as illustrated in FIG. 19 may be combined for metering. A metering mechanism not illustrated may be used. A more robust metering may be implemented by combining the plurality of metering mechanisms.

[0201]

(a) of FIG. 19 illustrates an example of the metering mechanism, which includes a vent filter 702 and a flow path 703 that connects with the flow path 328. The flow path 703 is connected to a dispensing chamber 320 side with respect to the valve 324 of the flow path 328. A material of the vent filter 702 is preferably a hydrophobic porous filter made of PP, fluorine-based resin, or the like. Steps I to III in FIG. 20 illustrate an operation example of the metering mechanism.

[0202]

Step I: a pressure is applied to the PCR chamber 304 to feed the PCR reaction solution into the dispensing chamber 320. Air present in the dispensing chamber 320 or in front of and behind the dispensing chamber 320 is released from the vent filter 702 via the flow path 703.

[0203]

Step II: the amplification products or the electrophoresis reagents cannot exceed the vent filter 702, and thus the PCR reaction solution is metered to the volume of the dispensing chamber 320 and the flow paths in front of and behind the dispensing chamber 320.

[0204]

Step III: the valve 337 is closed, and the valve 324 is opened. The PCR reaction solution in the dispensing chamber 320 is sent to the mixing chamber 327 by the electrophoresis reagent or air.

[0205]

As illustrated in (b) of FIG. 19, a flow path resistance 704 may be provided on the flow path 328. The flow path resistance 704 may be a hydrophobic filter made of PP, fluorine-based resin, or the like. In addition, the hydrophobicity of a flow path surface of the flow path resistance 704 may be higher than that of the flow path 328. In addition, a flow path width may be narrowed by the flow path resistance 704, or an obstacle may be provided. In addition, the flow path resistance 704 may have a rapidly widened flow path width. The flow path resistance 704 may be provided using a principle of a capillary stop valve. Air can easily escape until the flow path resistance 704 comes into contact with the liquid. Steps I to III in FIG. 21 illustrate an operation example of the metering mechanism.

[0206]

Step I: a pressure (A kPa) is applied to the PCR chamber 304 to feed the PCR reaction solution into the dispensing chamber 320. Air present in the dispensing chamber 320 or in front of and behind the dispensing chamber 320 can smoothly escape against the flow path resistance 704.

[0207]

Step II: at the applied pressure of A kPa, the PCR reaction solution cannot exceed the flow path resistance 704 or cannot exceed the flow path resistance 704 for a long time, and thus a specified amount of the PCR reaction solution can be metered in the dispensing chamber 320.

[0208]

Step III: the valve 337 is closed, and a pressure of B kPa (where $B > A$) is applied to the dispensing chamber 320 by the electrophoresis reagent or air. The PCR reaction solution

in the dispensing chamber 320 can be sent into the mixing chamber 327.

[0209]

As illustrated in (c) of FIG. 19, a liquid surface detection sensor 705 may be attached to the flow path 328 or the dispensing chamber 320, and the transport of solution may be stopped when the dispensing chamber 320 is filled with a specified amount of solution.

[0210]

FIG. 22 illustrates an example of a simple metering mechanism that does not use a liquid surface detection sensor, a vent filter, or a flow path resistance. This method may be combined with a liquid surface detection sensor, a vent filter, a flow path resistance, and the like to provide a robust dispensing mechanism.

[0211]

Step I: there is the PCR chamber 304 containing the PCR reaction solution 335, and m cycles of the PCR reaction are performed in the PCR chamber 304 with all the valves closed.

[0212]

Step II: after m cycles of the PCR reaction are completed, the valves 326 and 323 are opened, and a pressure in the PCR chamber 304 is set to the atmospheric pressure (= 100 kPa).

[0213]

Step III: the pressure of 100 kPa is applied through the flow path 319 and the flow path 330.

[0214]

Step IV: the valve 337 is opened, and a part of the solution in the PCR chamber 304 is transported to the dispensing chamber 320. When air in the dispensing chamber 320 is compressed to about half, the pressures in the dispensing chamber 320 and PCR chamber 304 are balanced, and the transport of the solution is stopped.

[0215]

Step V: the pressure in the dispensing chamber 320 is returned to the atmospheric

pressure by closing the valve 337 and opening the valve 324.

[0216]

In this method, when the solution is transported from the PCR chamber 304 to the dispensing chamber 320, the amount of solution that has entered the dispensing chamber 320 can be determined by the pressure applied inside the dispensing chamber 320. When the dispensing chamber 320 has a volume V_1 and is filled with air at a pressure P_1 , if the liquid enters the dispensing chamber by a volume V_2 , the pressure is changed to $P_2 = P_1 * V_1/V_2$ according to the Boyle's law. When P_2 is balanced with the applied pressure, the transport of the solution is stopped. In step I, when the pressure in the dispensing chamber is 100 kPa, if the volume of the dispensing chamber 320 is reduced to half, the pressures in the dispensing chamber and the PCR chamber both become 200 kPa, and are balanced. Therefore, the PCR reaction solution 335 corresponding to about 50% of the volume of the dispensing chamber can be dispensed. The pressure illustrated here is merely an example, and the volume of the dispensing chamber, the spaces of the flow paths in front of and behind the dispensing chamber, and the amount of solution to be metered may be appropriately set and metered. The disadvantage of this method is that the valve tends to require high-pressure resistance. If the volume of the dispensing chamber 320 is set to be larger than the amount to be metered, the pressure required for dispensing and metering decreases, but there is a disadvantage that the amount of remaining solution is increased in the case of being pushed out with the electrophoresis reagent.

[0217]

[Regarding Metering Accuracy]

In the CE analysis, if there is variation in the peak intensity when the same sample is measured a plurality of times, the effective analysis range is narrowed. When the analysis intensity greatly varies due to the introduction of the split PCR, the effective analysis range becomes narrow, which is not preferable. When the variation in the mixing ratio of the

electrophoresis reagent and the PCR reaction solution increases due to the introduction of the split PCR, the variation in the mixing ratio is reflected in the variation in the peak intensity, and the effective analysis range is narrowed.

[0218]

As illustrated in FIG. 14, when the PCR reaction solution and the electrophoresis reagent were mixed, how much the variation in the mixing ratio affects the peak intensity of CE was confirmed by an experiment. FIG. 23 illustrates the CE intensity when a ratio of the PCR reaction solution to the electrophoresis reagent was changed. The variation in the peak intensity between the capillaries was normalized by an average intensity of separately measured size standards containing no PCR reaction solution. The peak intensity of the size standard monotonically decreased as the ratio of the PCR reaction solution increased. On the other hand, the peak intensity of the amplification product monotonically increased, and the variation in the peak intensity was limited with respect to the variation in the amount of solution.

[0219]

In the CE measurement, when the amplification product is drawn into the capillary, a voltage is applied to the electrophoresis sample and the capillary to perform electric field injection. In the electric field injection, the amplification product introduced into the capillary is injected competitively with other ions and nucleic acids. When the voltage and an injection time are constant, a relation shown in Equation 1 is established (β is a current value, and α is a constant).

[Math. 1]

$$\alpha(k_0 * C_0 + k_1 * C_1 + k_2 * C_2 + k_3 * C_3) = \beta \quad \cdots \text{ (Equation 1)}$$

Where:

K_0 and C_0 : mobility and concentration of ions contained in the formamide,

K_1 and C_1 : mobility and concentration of size standard DNA,

K_2 and C_2 : mobility and concentration of salt, primer, and dNTP contained in the PCR reaction solution, and

K_3 and C_3 : mobility and concentration of the amplification product contained in the PCR reaction solution.

[0220]

When the amount of the PCR reaction solution varies by γ μ l with a case in which the amount of the PCR reaction solution is mixed at 1 μ l with respect to 10 μ l of the electrophoresis reagent, Equation 1 is expressed as follows.

[Math. 2]

$$\alpha * \left(k_0 * C_0 * \frac{11}{11 + \gamma} + k_1 * C_1 * \frac{11}{11 + \gamma} + k_2 * C_2 * (1 + \gamma) + k_3 * C_3 * (1 + \gamma) \right) = \beta$$

[0221]

An injection amount $k_1 * C_1$ of the size standard can be summarized as follows, which decreases monotonically with increasing γ .

[Math. 3]

$$k_1 * C_1 = \frac{\beta}{\alpha} - \left(k_0 * C_0 + \frac{11 + \gamma}{11} (1 + \gamma) (k_2 * C_2 + k_3 * C_3) \right)$$

[0222]

On the other hand, an injection amount $k_3 * C_3$ of the amplification product is as follows when Equation 1 is modified, which monotonically increases with respect to the amount of the PCR reaction solution.

[Math. 4]

$$k_3 * C_3 = \frac{\beta}{\alpha} - \left(k_2 * C_2 + \frac{11}{(11 + \gamma)(1 + \gamma)} (k_0 * C_0 * + k_1 * C_1) \right)$$

[0223]

The PCR reaction solution contains many salts, primers, dNTPs, and the like, and the injection amount is almost saturated. Therefore, even if the proportion of the PCR reaction solution increases slightly with respect to the electrophoresis reagent, the variation in the peak intensity is limited. Even if the ratio of the PCR reaction solution varies by $\pm 20\%$ with respect to standard conditions (PCR reaction solution: electrophoresis reagent = 1: 10), a variation rate of the peak intensity is kept within 10%. In addition, if a condition under which the PCR reaction solution is slightly darker than the standard conditions (PCR reaction solution: electrophoresis reagent = 1.6: 10) is targeted, even if the ratio of the PCR reaction solution varies by $\pm 50\%$ from a target ratio, the variation rate of the peak intensity becomes 10% or less. This experiment result suggests that even if the dispensing accuracy of the split PCR is somewhat poor, the variation in the peak intensity derived from the amplification product is limited due to the variation in the mixing ratio of the PCR reaction solution and the electrophoresis reagent.

[0224]

Even if the accuracy of the metering mechanism is somewhat poor, it may be preferred to adopt a simple and low-cost metering mechanism. When a metering mechanism with low accuracy is adopted, as described above, it is preferred to select solution compositions in which a variation in the mixing ratio of the PCR reaction solution and the electrophoresis reagent is less likely to affect the peak intensity. This can be achieved by adding salt to the electrophoresis reagent, or by using salts, primers, dNTPs, and the like contained in the PCR reaction solution. In addition, the mixing ratio of the electrophoresis reagent and the PCR reaction solution is performed under such a condition that the PCR

reaction solution is slightly larger, and is measured in a state closer to saturation, thereby making it difficult to be affected by the variation ratio. In this case, it is preferred to mix the size standards slightly larger than a standard mixing ratio such that the peak intensity of the size standard contained in the electrophoresis reagent reaches a level necessary for measurement.

[0225]

<<Method of Setting Split PCR Protocol>>

[Principle of Expansion of Analysis Range by Split PCR]

[0226]

In order to enable the analysis of target DNA of various concentrations contained in a sample, the PCR reaction solution m and the PCR reaction solution n containing amplification products having different concentrations are subjected to the electrophoresis analysis, respectively. By setting m and n in advance from an appropriate range in accordance with a concentration range of the target DNA that may be contained in the sample, an appropriate DNA profile can be obtained for the amount of DNA in a wider range than in the case of preparing one amplification product and performing CE analysis without lowering the sensitivity.

[0227]

FIG. 24 illustrates an expansion principle of the analysis range by the split PCR. In FIG. 24, a horizontal axis represents the amount of DNA input to the PCR, and a vertical axis represents the concentration of the amplification product or the peak intensity in the CE. Reference numeral 801 indicates a CE detection upper limit or an upper limit of the amplification product concentration at which the PCR can be accurately amplified, and reference numeral 802 indicates a CE detection lower limit. In FIG. 24, the concentration of the amplification product is expressed to linearly increase with respect to the amount of input DNA, but it is considered that an actual amplification is in a sigmoid shape and approaches a

plateau when the amplification product reaches a certain amount. It is assumed that for a certain number m of PCR cycles, a range of the amount of DNA that can be correctly analyzed is between a lower limit a and an upper limit b . The analysis range of the STR-CE that can be analyzed in one cycle number is 803. In addition, it is assumed that for a certain number n of PCR cycles, a range of the amount of DNA that can be correctly analyzed is between a lower limit c and an upper limit d . At this time, if $a < d$, the analysis range can be expanded from b/a or d/c to b/c . It is assumed that the analysis range of the STR-CE when being expanded by the split PCR is 804. It is assumed that 804 is a range of the amount of DNA in which either m or n falls within the analysis range of the STR-CE, and in principle, there is no amount of DNA, which falls outside the analysis range, within the range. Hereinafter, it is assumed that the analysis range (b/a) in each cycle number is substantially constant and changes 2^x times with respect to a cycle number difference x . At this time, an interval between m and n is limited to a range of the following Equation 2.

[Math. 5]

$$n - m \leq \log_2 \left(\frac{b}{a} \right) \quad (n \text{ and } m \text{ are integers}) \quad \cdots \quad (\text{Equation 2})$$

[0228]

An example of a case in which Equation 2 is satisfied is illustrated in (1) of FIG. 24. For example, when the analysis range b/a with respect to the amount of DNA brought into the PCR is 80 times, $n - m$ is suitably 6 or less, and an expansion rate is 64. The expansion rate can be increased by $(b/a)^{y-1}$ with respect to a division number y , but it is considered that the smaller the division number, the more stable transport of solution can be implemented on a simple flow path device. Hereinafter, a case in which m and n are set and the amplification products m and n are analyzed will be mainly described. On the other hand, by using the same setting method, l may be set to be divided into three, and k may be set to be divided into four.

[0229]

When c or d is less than 0.2 ng or 0.1 ng, a variation in an intensity balance between peaks increases, and thus it is preferred to set the number of cycles larger than the number of cycles set by Equation 2 and to set the interval between m and n narrower than the maximum number of cycles defined by Equation 2. Hereinafter, unless otherwise specified, it is assumed that an upper limit and a lower limit of analysis of the STR-CE change 2^x times with respect to the cycle number difference x .

[0230]

(2) of FIG. 24 illustrates the analysis range when the interval between m and n does not satisfy Equation 2. In this case, the lower limit a that can be analyzed with m exceeds the upper limit d that can be analyzed with n , and correct DNA test cannot be performed with a DNA concentration between a and d . If $a > d$ and there is no sample between a and d , or if a frequency of such a sample is extremely low, values of m and n may be set such that the relation $a > d$ is established, but the amount of DNA in the sample to be input actually is often unknown, and thus it is preferred to satisfy Equation 2.

[0231]

When DNA with an extremely low concentration is covered with the number of cycles n , a stochastic effect occurs, and thus it is desirable to set a difference to be smaller than an upper limit of the difference between n and m specified by Equation 2.

[0232]

The STR-CE analysis has factors that cause a variation in a plurality of analysis results, and thus it is necessary to provide an overlap between the analysis range of the amount of DNA can be covered by m cycles of STR-PCR and a range of the amount of DNA that can be analyzed by n cycles of STR-PCR taking into consideration of the variation. If there is no overlap, there is the amount of DNA in which analysis fails even though the amount of DNA is within the analysis range of m and n . That is, it is necessary to set m and n such that

$a < d$ is always established for an assumed analysis variation. Hereinafter, it is assumed that m and n are set taking into consideration of the variability in the analysis system.

[0233]

[Setting n according to CE Analysis Results of m]

A numerical value of m may be preset in the device.

[0234]

In the case of the analysis system in which step 505 starts or ends after step 504, n may be set according to a measurement result of the electrophoresis sample m .

[0235]

n may be set according to the following conditions:

(1) when no peak of the amplification product m is detected at all,

(2) when peaks are detected and a part of the peaks are below the AT, and

(3) when peaks are detected, and a part of the peaks are already saturated or exceed an IAP threshold.

[0236]

In the case of (1), if the analysis range of the STR-CE is x , $n - m$ cycles ($n - m \leq \log(x)$, where x is the analysis range of the STR-PCR) may be performed additionally. In this case, it is preferred to use a maximum value of $n - m$ so as to satisfy Equation 2.

[0237]

In the case of (2), when a CE saturation intensity is z with respect to a maximum intensity y of the detected peak, the PCR specified by $n - m$ cycles ($n - m \leq \log(z/y)$) may be performed additionally.

[0238]

In the case of (3), the additional PCR may not be performed.

[0239]

[Method of Setting Interval between m and n Taking into Consideration of Intensity Ratio of

Samples to be Analyzed]

In particular, when a time taken for the CE analysis is long, waiting until the m cycles of electrophoresis as described above are completed, a waiting time for $n - m$ cycles of the PCR reaction is too long, and thus the artifacts may increase or the PCR reaction may not be successfully performed. Therefore, m and n may be set in advance.

[0240]

The STR-CE contains a mixture of a homozygous locus and a heterozygous locus, and thus even in an ideal analysis system in which all the amplification efficiency, the CE injection efficiency, and the light emission efficiency of the fluorescent dye are equal, a difference in intensities between the heterozygous peak and the homozygous peak is 1: 2.

[0241]

In actual STR-CE, there are a difference in the amplification efficiency, a difference in the CE injection efficiency, and a difference in the light emission efficiency between dyes depending on a locus and a DNA length, and thus even if the amount of DNA is appropriately adjusted, a difference in intensities between peaks is 1: 2 to 1: 20. The AT may be set to different values between the dyes. In this case, it is necessary to consider the analysis range taking into consideration of a difference between the setting values of the AT.

[0242]

When DNA is derived from a plurality of persons, there is a difference of 1: 2 to 1: 1,000 in the abundance ratio of alleles.

[0243]

When DNA is deteriorated, there is a difference of 1: 2 to 1: 1000 in the abundance ratio of alleles. Typically, a large amount of DNA from a short locus or an allele is amplified, and the peak intensity of DNA from a long locus or an allele tends to decrease.

[0244]

When the PCR is inhibited, the short DNA is more amplified, and the peak intensity

of the long DNA tends to decrease. In such a case, a difference in the peak intensities is 1: 2 to 1: 1000.

[0245]

When the amount of input DNA is less than 0.1 ng, the intensity variation between peaks tends to increase. In this case, the effective analysis range is further reduced. Therefore, effective dynamic ranges that can be covered by m and n are not necessarily the same.

[0246]

Hereinafter, the discussion will be made on the assumption that a factor for determining the intensity ratio between the peaks is derived from the difference in the abundance ratio of the alleles contained in the DNA input to the STR-PCR. The difference in the amplification efficiency, the difference in the CE injection efficiency, and the difference in the light emission efficiency are also affected, and thus the differences should be originally taken into consideration, but is omitted, and is included in the discussion of the abundance ratio between the alleles.

[0247]

An effective analysis range of the STR-CE in the case in which there is a difference in the abundance ratio of the alleles to be analyzed will be described with reference to FIG. 25. The amplification product contains an allele α and an allele β . The amounts of the alleles α and β are $\alpha > \beta$ before or after the amplification. A plot of the amount of amplification products with respect to the amount of DNA input from the allele α , or the peak intensity of CE is defined as a plot 805. A plot of the amount of amplification products with respect to the amount of DNA input from the allele β , or the peak intensity of CE is defined as a plot 806. The plots 805 and 806 are not necessarily straight lines. An amplicon α can be analyzed in a range of the amount of input DNA a to b. In addition, an amplicon β can be analyzed in a range of the amount of input DNA c to d. If the PCR amplification is linear in

the concentration range of a to b ,

[Math. 6]

$$c = a * \frac{\alpha}{\beta}$$

is established. The effective analysis range 803 of the STR-CE in which both the amplicons α and β are correctly detected can be expressed by the following Equation (3).

[Math. 7]

$$J05 = \frac{b}{a} * \frac{\alpha}{\beta} \quad \cdots \quad (\text{Equation 3})$$

b/a (range 807) may correspond to the dynamic range of the CE, or may be the analysis range of the STR-CE in which a peak α can be detected.

[0248]

The effective analysis range becomes smaller as the intensity ratio of the DNA to be analyzed increases, as shown in Equation 3.

[0249]

FIG. 26 illustrates a correspondence table of an intensity ratio α/β of a maximum amplicon to a minimum amplicon that can be analyzed by the split PCR with respect to the numerical value corresponding to the range 807 and the setting value of $n - m$. For example, when the range 807 is set to 120 and $n - m$ is set to 5, an allele having an abundance ratio of $1/3.75$ with respect to an allele having a highest abundance ratio is to be analyzed. On the other hand, when an intensity ratio of an allele set to be analyzed is larger than a ratio illustrated in FIG. 26, this means that the concentration at which the maximum peak or the minimum peak conflicts with the upper limit or the lower limit of analysis appears inside the analysis range. For example, as illustrated in (1) of FIG. 27, there is no problem if both α and β can be detected at certain concentrations, but as illustrated in (2) of FIG. 27, a situation may occur in which only α can be detected and β cannot be detected.

[0250]

The peaks having too large a peak intensity ratio may not be analyzed. An example is shown below.

·The STR-CE includes the stutter peaks, and peaks having a peak intensity separated by 1: 20 or more in one locus are less likely to be distinguished from the stutter peaks, and thus peaks having an intensity ratio larger than 1: 20 or 1: 40 in one locus may not be analyzed.

·The total amount of deteriorated DNA may be small, and thus peaks having peak intensities separated by 1: 20, 1: 40, or 1: 100 or more may not be analyzed.

·As described above, in an electropherogram in which the intensity ratio between peaks is extremely large, a small peak is not significant, and thus a threshold of the inter locus PHR that defines the minimum peak intensity with respect to the maximum peak intensity to be analyzed may be set. When the interval between m and n is set using FIG. 27, it is preferred to set the abundance ratio of the alleles to be analyzed to be slightly smaller than the threshold of the inter locus PHR.

[0251]

[Setting Method of n]

When the partial profile is also an analysis target, 30 to 34 cycles are appropriate.

[0252]

The peak derived from one copy of DNA can be detected by setting the number of cycles to 36.

[0253]

When the number of cycles exceeds 36, a detection probability of a peak derived from a person is not improved although the number of artifacts (particularly, a frequency of occurrence of a stutter or drop in remarkably increases) increases, and thus it is not preferred to set the number of cycles to 36 or more.

[0254]

When the partial profile is not to be analyzed, 30 or 29 cycles or less are appropriate.

[0255]

Regarding the sample containing the minimum amount of DNA, it is preferred to select a minimum cycle number with a smallest number of error determinations, which may be examined in an experiment.

[0256]

The n cycles may be set to the minimum number of PCR cycles at which a peak of the amplicon derived from one copy always exceeds the AT.

[0257]

The n cycles may be set to the minimum number of PCR cycles at which the amplification product derived from 20 copies of genomic DNA always becomes the full profile.

[0258]

The n cycles may be set to the maximum number of PCR cycles at which the intensity ratio of stutter peaks or other peaks due to an amplification error (excluding IAP) does not exceed a threshold.

[0259]

After the detection lower limit in a specific number of cycles is made clear by an experiment or calculation, n may be set under the assumption that the detection lower limit increases or decreases twice for each cycle. When the amount of DNA is too small, the variation in the peak intensity becomes larger due to the stochastic effect, and thus it is more preferred to set the number of cycles with a slight margin.

[0260]

n may be set by using the above method independently of m. If the interval between m and n is too large, the amount of DNA that cannot be analyzed may remain between m and n.

[0261]

If m is set in advance, n may be set based on a difference $n - m$ that can cover the intensity ratio of the peaks to be analyzed, and the numerical value of m as illustrated in FIG. 26.

[0262]

When the analysis range 803 is known, it is preferred to set n with a cycle number difference of $\log_2$ (analysis range 803) or less with respect to m .

[0263]

The number of cycles may be set with a margin, taking into consideration of a fluctuation in various analysis systems or the peak intensity ratio.

[0264]

[Setting Method of m]

The DNA to be input to the PCR hardly exceeds 1.5 μg in the DNA test, and thus even if the number of cycles is set to be less than 20, the number of loci that can be correctly detected does not increase. Therefore, the number of cycles is preferably set to 20 or more.

[0265]

It is preferred that the amount of DNA that may be input to the PCR is evaluated based on a sample containing the maximum amount of DNA that may be brought into the analysis system, and when the DNA is subjected to the STR-CE analysis, the number of cycles is set to the maximum number of PCR cycles in which no OS or IPA flag appears.

[0266]

When the maximum amount of DNA to be input to the STR-CE is determined, it is more preferred to set the maximum number of cycles that can satisfy 201 to 207 in FIG. 2 when the amount of DNA is input.

[0267]

The number of cycles may be set with a margin, taking into consideration of the

fluctuation in various analysis systems or the peak intensity ratio.

[0268]

The detection lower limit in a specific number of cycles, the amount of DNA to be input to the PCR, and the amount of DNA in the detection upper limit may be examined, and m may be set under the assumption that the lower limit and the upper limit increase or decrease twice per cycle.

[0269]

m may be set by using the above method, independently of n . If the interval between m and n is too large, the amount of DNA that cannot be analyzed may remain between m and n .

[0270]

If n is set in advance, m may be set based on the difference $n - m$ that can cover the intensity ratio of the peaks to be analyzed, and the numerical value of n as illustrated in FIG. 26.

[0271]

When the analysis range 803 is known, it is preferred to set m with a cycle number difference of $\log_2$ (analysis range 803) or less.

[0272]

Even if the interval between m and n is 2 or 3, the expansion rate of the analysis range is kept at four times or eight times. In order to perform the DNA test with high robustness, it is desirable to expand the analysis range by one digit or more. Therefore, it is preferred that the interval between m and n is set to 4 or more. As described above, in the preferred split PCR, a maximum setting value of n is 36 and a minimum setting value of m is 20, and thus a minimum setting value of n is 24 and a maximum setting value of m is 32.

[0273]

[Setting m and n based on Table]

(1) of FIG. 28 illustrates the expansion rate of the analysis range when m and n are set. Based on this table, the interval between m and n may be determined to obtain a necessary expansion rate. This table does not consider a change in the analysis range depending on the DNA concentration range such as the stochastic effect. In addition, when an original analysis range is larger than the expansion rate, a range of the amount of DNA that cannot be analyzed is generated as illustrated in (2) of FIG. 24, which is unsuitable. In addition, when DNA having large differences in the abundance ratio between the alleles is to be analyzed, if the expansion rate is set to the limit of the original analysis range, there is an allele that cannot be analyzed.

[0274]

(2) of FIG. 28 illustrates (the number of digits of) the analysis range expanded by the split PCR. In a typical STR-CE, 0.75 ng to 48 ng (1.8 digits) of genomic DNA can be analyzed during 25 PCR cycles. This range varies depending on the personal DNA, depending on the quality of DNA, and depending on the CE measurement system. Therefore, this range needs to be evaluated for each measurement system. In addition, the variability also needs to be taken into consideration. Based on this result, the number of digits of the analysis range when m and n are changed, the analysis lower limit of n cycles, and the analysis upper limit of m cycles are shown. The stochastic effect when the amount of DNA was reduced was not taken into consideration. The table was prepared by changing all range by 2^n with respect to the number of cycles. One like this table may be created, and m and n may be determined based on appropriate lower and upper limits and the analysis range. In this case, when the expansion rate of the analysis range exceeds 1.8 digits, as illustrated in (2) of FIG. 24, there is a range of the amount of DNA that falls outside the analysis range within a section considered to be the analysis range.

[0275]

A typical dynamic range of the CE is 2,000 or less. The peak intensity ratio becomes

twice or more even in the ideal DNA test, and thus the difference between n and m is preferably 9 or less.

[0276]

The dynamic range of the CE with higher performance is 4,000 or less. The peak intensity ratio becomes four or more even in many DNA tests, and thus the difference between n and m is preferably 9 or less.

[0277]

As described above, in an example of the preferred range, m is 20 or more and 32 or less. In addition, in an example of the preferred range, n is 24 or more and 36 or less. Further, in an example of the preferred range, n is larger than m by 4 to 9.

[0278]

[Timing of Division]

As illustrated in FIG. 14, the final extension step may be performed and divided after m thermal cycles are completed, and the final extension may be performed again after n thermal cycles are completed. In this case, for example, the analysis system 101 maintains the PCR reaction solution m at a constant temperature within a range of 50°C to 80°C for 1 minute to 20 minutes, and then takes out a part of the PCR reaction solution m. With this configuration, only one heater is required around the PCR, and thus the device or the structure of the flow path device becomes simple.

[0279]

The final extension step may be performed after the division after m thermal cycles are completed.

[0280]

FIG. 29 illustrates an excerpt of a method of using the analysis system 101. The description of parts common to FIG. 14 may be omitted. Steps 601 to 612 illustrated in FIG. 29 may correspond to examples of detailed steps from step 404 (amplification of sample) to

step 405, and steps 601 to 612 illustrated in FIG. 29 may be processes independent of steps 404 to 405.

[0281]

The operation as illustrated in FIG. 29 can be performed in the flow path device 104 of FIG. 6. In FIG. 6, a heating unit 318 is in contact with the dispensing chamber 320. After m cycles are completed in step 603, the division is performed by the dispensing chamber 320 in step 605, and the final extension step is performed by the heater in step 604. The PCR reaction solution remaining in the PCR chamber may be subjected to n - m thermal cycles in step 608 in parallel with step 604, or may be performed at different times. After n thermal cycles, the product n may be subjected to the final extension step in a holding chamber. That is, in the example of FIG. 29, for example, the analysis system 101 maintains the part of the PCR reaction solution m at a constant temperature within the range of 50°C to 80°C for 1 minute to 20 minutes.

[0282]

As an advantage of performing the operation after the division, the product n does not experience the final extension step twice, and thus the artifacts are reduced and the entire time is not lengthened.

[0283]

[Method for Providing CE Analysis Results to User]

When the CE is performed twice or more in the split PCR, two or more electropherograms are generated. Both the two electropherograms or the DNA test results may be provided to the user. Whether two or more electropherograms are scored as more preferable for the DNA test may be provided together with the CE analysis results.

[0284]

For example, the analysis system 101 may determine which of a result of the electrophoresis analysis on the part of the PCR reaction solution m and a result of the

electrophoresis analysis on the at least part of the PCR reaction solution n is a better result, and output the better result. Alternatively, information capable of determining which is the better result may be output. In addition, it may be known whether the full profile is obtained using a part of the flowchart illustrated in FIG. 2. The scoring may be performed using the number of loci that satisfy the determination references illustrated in FIG. 2, the number of loci that do not satisfy the determination references, or the number of flags that are determined not to satisfy the determination references, or the scoring may be comprehensively calculated by an algorithm based on the determination references. Data provided to the user may be data in the middle of the DNA test analysis. It is also possible to provide the user with only the analysis result in which the full profile is obtained or the analysis result determined to be a better electropherogram. In this way, the comparison of the results can be performed efficiently. In addition, when the non-experts receive data from the system, an appropriate result is likely to be selected.

[0285]

When both the analysis results are bad, a review made by the experts may be requested.

[0286]

The two pieces of data can also be combined for the DNA test. In particular, in the DNA test in which the peak intensity ratio is large, it can be assumed that an allele indicating the minimum intensity is less than the AT at m, and an allele indicating the maximum intensity undergoes oversaturation at n. In this case, significant peaks or DNA test results can be extracted from m and n, respectively, and synthesized and provided. That is, the present embodiment can be used to expand the dynamic range of the CE.

[0287]

The number of times that the CE measurements are performed in the analysis system may be two, one, or even three or more.

[0288]

Depending on the state of the sample, the analysis may be performed only once, twice, or more times. As compared with a case in which only one PCR product is prepared every time and the CE analysis is performed only once, a correct DNA test result may be obtained, and it is not necessary to perform measurement many times every time, and thus the analysis at low cost and in a short time can be performed, and the throughput can be improved.

[0289]

When the analysis is performed only once, either the amplification product m or the amplification product n may be measured.

[0290]

In the case of performing the measurement twice, the product of m cycles may be measured twice, the product of n cycles may be measured twice, the product of n cycles may be measured after m cycles, the analysis of m cycles may be performed after n cycles, the analysis of n cycles may be started regardless of a state of data of m cycles, the analysis of m cycles may be started regardless of a state of data of n cycles, or the analysis of m and n may be performed completely simultaneously.

[0291]

If a first electrophoresis result is electrophoresis failure, the amplification product of the same number of cycles may be analyzed again. In addition, if the first electrophoresis result is electrophoresis failure, the product of the other number of cycles may be analyzed. Here, the electrophoresis failure refers to a situation in which a part or all of the size standard peaks cannot be detected, or a situation in which the size standard peaks are caught somewhere during the transport of the solution.

[0292]

After the analysis result of either the amplification product m or n is obtained, the analysis of the other amplification product may be started or continued based on a feedback

obtained by collating the obtained DNA test result with a database.

[0293]

The user may determine whether to analyze, after the analysis result of either the amplification product m or n is obtained, the other amplification product. The user may perform a determination based on first data or analysis score, or may determine whether to perform a second measurement at any time regardless of the data. The product m or n may be held inside or outside the device, taken out from a cartridge or the analysis system 101 after the measurement is once completed, and measured outside the device. In addition, the product m or n may be stored in the cartridge for a certain period of time and then measured again in the device. During this time, the amplification product is preferably stored in a refrigerated or frozen state. The amplification product may be mixed with the electrophoresis reagent and stored in the state of the electrophoresis sample, or may be stored in the state before being mixed with the electrophoresis reagent.

[0294]

<When Amplification Product m is Analyzed First>

When the amplification product m is analyzed first, it may be determined whether to analyze the amplification product n according to a flowchart illustrated in FIG. 30. However, the flowchart illustrated in FIG. 30 is an example, and may include the determination references or branching conditions not shown here. For example, the determination references may be changed according to the obtained number of peaks, the number of loci, whether data is mixed, and the like. In addition, the determination references illustrated in FIG. 30 may be partially or entirely omitted, or may be replaced with other references.

[0295]

An electrophoresis result of m may be provided to the user to determine whether to analyze the product n and then start.

[0296]

In addition, when n are being analyzed simultaneously or in parallel, the analysis may be interrupted midway based on the determination result of the user or the flowchart of FIG. 30.

[0297]

Determination set 1: whether there is a peak that saturates the CE detection system. If there is a peak, the analysis of the amplification product n is not performed or interrupted.

[0298]

Determination set 2: is the full profile obtained? If the full profile is obtained, the analysis of the amplification product n is not performed or interrupted.

[0299]

Determination set 3: does the IAP+ flag appear? If the IAP+ flag appears, the analysis of the amplification product n is started or continued.

[0300]

Determination set 4: are all peaks $1/2$ or less of the OS intensity? or are all peaks equal to or less than an intensity obtained by dividing the OS according to the expansion rate expected by the split PCR? or are all peaks equal to or less than an intensity at which saturation does not occur when additional PCR cycles n - m are performed? or do all peaks not reach any peak intensity? If all peaks reach any peak intensity, the analysis of the amplification product n is not performed or interrupted.

[0301]

When the interval between m and n is 2, if almost no peaks are detected in the CE analysis of the electrophoresis product m, significant data cannot be obtained even with the electrophoresis product n, and thus it is not significant to analyze n after the analysis result of m is obtained. Similarly, when the interval between m and n is narrow, the analysis of n may not be performed if it is determined based on the analysis result of m that the amount of DNA is too small. When it is determined whether the amount of DNA is too small, the number of

detected peaks may be used, or the peak intensity may be used.

[0302]

In the various determination sets, different determination reference sets may be used depending on whether each of the loci is a heterojunction, a homojunction, a mix, or a single.

[0303]

When the IAP- peak exceeds the threshold, n cycles of analysis may be performed. The IAP- peak is generated when the amount of input DNA is only excessively small for the PCR cycles, and thus it is considered that the IAP- peak can be reduced by increasing the number of cycles.

[0304]

In addition, when it is determined based on the CE analysis result of m that a significant CE analysis result cannot be clearly obtained even if the analysis of n is performed, various thresholds or determination algorithms may be provided so that it can be determined that the analysis of n is interrupted or is not performed.

[0305]

<When Amplification Product n is Analyzed First>

When the amplification product n is analyzed first, it may be determined whether to analyze the amplification product m according to a flowchart illustrated in FIG. 31. However, the flowchart illustrated in FIG. 31 is an example, and may include the determination references or branching conditions not shown here. For example, the determination references that change according to the obtained number of peaks, the number of loci, whether data is mixed, and the like can be assumed. In addition, the determination references illustrated in FIG. 31 may be partially or entirely omitted, or may be replaced with other references.

[0306]

An electrophoresis result of n may be provided to the user to determine whether to

analyze the product m and then start.

[0307]

In addition, when m are being analyzed simultaneously or in parallel, the analysis may be interrupted midway based on the determination result of the user or the flowchart of FIG. 30.

[0308]

Determination set 1: whether there is a peak that saturates the CE detection system. If there is a peak, the analysis of the amplification product m is started or continued.

[0309]

Determination set 2: does the IAP+ flag appear? If the IAP+ flag does not appear, the analysis of the amplification product m is not performed or interrupted.

[0310]

In addition, when it is determined based on the CE analysis result of n that a significant CE analysis result cannot be clearly obtained even if the analysis of m is performed, various thresholds or determination algorithms may be provided so that it can be determined that the analysis of m is interrupted or is not performed.

[0311]

In this way, the analysis system 101 subjects one of the part of the PCR reaction solution m and the at least part of the PCR reaction solution n to the electrophoresis analysis in the CE unit 105, and controls execution of the electrophoresis analysis on another one of the part of the PCR reaction solution m and the at least part of the PCR reaction solution n based on a result of the electrophoresis analysis on the one. For example, it may be determined whether to start the other electrophoresis analysis based on the result of the one electrophoresis analysis, or it may be determined whether to continue the other electrophoresis analysis based on the result of the one electrophoresis analysis after the other electrophoresis analysis is started. In this way, unnecessary and inefficient electrophoresis analysis is

omitted, and the entire processing is efficiently performed.

[0312]

[Both of n and m are Preset]

The analysis system 101 may be preset with two different numbers of cycles, and n and m. As described above, m and n are preferably set appropriately in accordance with the CE analysis range or the amount of DNA that can be amplified.

[0313]

Depending on the type of samples to be measured, different sets of n and m and an analysis protocol may be set as shown in Table 3.

[0314]

[Table 3]

Table 3 Examples of Preset Modes

	m	n	Measured first
Cheek swab mode	26	-	-
DVI mode	25	31	From m
Casework	26	31	From n
Touch sample	28	32	From n

[0315]

For example, when a large amount of DNA is stably contained to a certain extent as in a case of cheek swab, the STR-CE analysis may be performed in 26 cycles by selecting the "cheek swab mode" and without performing the division (or by performing only one CE analysis even if the division is performed and analyzing the other amplification product only when the division fails). In addition, a "disaster victim identification (DVI) sample mode" may be provided for performing DVI samples (DNA test to identify bodies left behind in disasters or large-scale terrorist attacks). The DVI sample is likely to contain a relatively large amount of DNA, and thus is set to, for example, $[m = 25, n = 31]$. In many cases, the

DVI sample contains a relatively large amount of DNA, and thus the analysis of m cycles may be started first, and the analysis of the amplification product n may be started or continued in a determination flow as illustrated in FIG. 30. In addition, when DNA obtained at the crime scene is analyzed, the amount of DNA is frequently small, and thus a "casework sample mode" in which $[m = 26, n = 31]$ is set may be selected. In the case of casework sample, the probability that the amount of DNA contained is extremely small is high, and thus the analysis of n can be started first, and a determination is made in which the analysis of m is performed or continued in the determination flow illustrated in FIG. 31. In addition, in the case of touch sample, there is a high possibility that the amount of DNA contained is very small, or the variation in the peak intensity is large due to deterioration, or the DNA becomes mixed DNA. Therefore, it is preferred that the interval between m and n is set narrower in the "touch sample mode" than in other modes. In addition, there is a high probability that the amount of DNA contained is small, and thus it is preferred to analyze n samples.

[0316]

When there are two or more CE in one sample, m and n may be analyzed simultaneously. In addition, the preset m and n and a measurement order may be changed by the user each time. The numerical values of m and n shown in Table 3 are merely examples, and the preset m and n may be set such that a validation test is performed at the time of development and a probability that the DNA test is most successful is highest.

[0317]

[Point of Present Embodiment]

In the above embodiments, a case in which both m and n are analyzed each time and a case in which only one of m and n is analyzed each time are described.

[0318]

Even if one of the amplification products prepared in two cycles cannot provide data with a required quality, a possibility that the data with the required quality can be obtained

from the other amplification product is higher than a case in which the amplification product is prepared in one cycle.

[0319]

Even if one of the amplification products prepared in two cycles cannot provide the data with the required quality, a probability that the sample is wasted can be reduced because the other amplification product is prepared. In addition, the probability that the sample is wasted is reduced, and thus it is possible to save time and effort for collecting and preprocessing the sample again. As a result, an average data acquisition time is shorter when two samples are prepared and analyzed using the present embodiment than when the present embodiment is not used.

[0320]

When the PCR products of m cycles are divided, the remaining PCR products are not subjected to PCR cycle, and only the PCR products of m cycles are subjected to the CE analysis, if the quality required for the CE analysis result of the PCR products of m cycles is not satisfied, the remaining PCR products can be subjected to $n - m$ PCRs to prepare PCR products of n cycles. When the PCR products are left standing for a waiting time for the CE analysis of the electrophoresis sample m , the activity of the polymerase decreases, or a large number of artifacts increase depending on a standing temperature, and thus the CE analysis result of the PCR products of n cycles also cannot satisfy the required quality.

[0321]

When only the PCR products of n cycles are prepared and only the PCR products of n cycles are subjected to the CE analysis, the samples are wasted if the PCR products of m cycles are not prepared when the CE analysis result of the PCR products of n cycles does not satisfy the required quality.

[0322]

When a DNA solution used for the PCR reaction is divided before the PCR, the

amount of DNA input to each of the divided DNA solutions decreases, and thus the sensitivity decreases. On the other hand, after the PCR reaction has progressed to a certain extent (for example, four cycles or more), 10 or more amplicons are contained in one allele, and thus the division does not affect the sensitivity.

[0323]

The division is preferably performed at a timing when m cycles are completed. When the division is performed before the m cycles are completed and the remaining PCR reaction is performed such that the total of m cycles is performed in each chamber after the division, it is necessary to install a PCR temperature controller in each chamber, and thus the size of the apparatus increases. In addition, an artifact may occur at the time of division, and thus it is preferred to perform division after the number of cycles becomes as large as possible.

[0324]

By preparing a plurality of electrophoresis samples having different dilution rates after the PCR, an effective analysis range of CE can be expanded. That is, the occurrence of oversaturation of the CE or a frequency of the peak intensity falling below the AT can be reduced. A problem of peak strength balance or peak cracking caused by too many amplification products is not solved by the dilution after the PCR, and needs to be dealt with before the PCR or during the PCR. That is, the split PCR is appropriate.

[0325]

The number of cycles for division is appropriately set for the analysis sample, and thus a maximum expansion rate of the analysis range is obtained with a minimum division number. That is, the measurement time at the time of expanding the analysis range is the minimum.

[0326]

By controlling the amount of DNA before being input to the PCR, the effective analysis range can be expanded. For example, the upper limit of the amount of DNA

adsorbed on the purified film can be reduced by changing a capacity or volume of the purified film, a purification protocol, and the like. However, in a typical purified film, if the volume is reduced in order to lower the upper limit of adsorption, a degree of passage of the solution becomes worse, or the yield of DNA (particularly, the deteriorated DNA) decreases. Therefore, the control of the amount of DNA in a purification stage is limited. By quantifying the amount of DNA before the PCR, the effective analysis range can also be expanded by controlling the number of PCR cycles or changing the dilution rate. However, in the quantification step, an additional detection system is required, and the flow path device cannot be complicated. There is a possibility of analysis failure due to a quantification error, and thus the split PCR is more preferable.

[0327]

The dilution after the PCR and the control of the amount of purified DNA may be performed in combination with the present embodiment. By combining these, it is possible to more reliably perform the DNA analysis on the amount of DNA in a wide range.

[0328]

By taking out the PCR reaction solution every cycle or skipping one cycle and subjecting the PCR reaction solution to the CE measurement, the DNA analysis suitable for all DNA concentration ranges can be performed. However, it is difficult to provide a mechanism in which a solution is taken out three times or more, four or more times, or five or more times in the flow path device, and then mixed with an electrophoresis reagent. In addition, even when two, three, or four amplification products are measured every cycle or skipping one cycle, the expansion rate of the analysis range is kept at two times, four times, and eight times, and is not necessarily suitable for analysis of a sample containing various amounts of DNA. In addition, in the STR-PCR, when the final extension is performed in the same chamber as the PCR chamber, if the interval between m and n is narrow (for example, one cycle or two cycles), the final extension time may be excessive depending on the amount

of DNA input to the PCR, and a large number of A++ peaks may appear. In particular, in the analysis system in which no heating unit 318 is provided, in a split PCR device in which final extension is performed in the same chamber as in FIG. 14, when the interval between n and m is narrow, a large number of A++ peaks appear, and thus two or more peaks are preferable.

[0329]

[Flowing Method according to Number of Capillaries]

i) When only one capillary is used for one sample,

FIG. 32 illustrates a typical example of an operation timing of the analysis system 101. In this operation timing, first, a first sample A is input in step 401, the sample is processed in a pretreatment cartridge in steps 402 to 404, and the electrophoresis sample m or n is analyzed by the CE in step 405.

[0330]

An operation procedure as illustrated in FIG. 32 is suitable for a case in which there is one CE unit for one sample pretreatment unit. Such a configuration makes the device small and convenient to carry.

[0331]

The data may be provided to the user at a timing when first CE analysis is completed in step 405. The user may determine whether the analysis of a second electrophoresis sample is started or continued. In addition, the user may determine whether the analysis of the second electrophoresis sample is started or continued by using the above determination method. This is because when the measurement is performed twice each time, the measurement time of the CE is doubled, and thus the throughput does not increase.

[0332]

The pretreatment of a sample B (steps 401 to 404) may be started at a timing when the pretreatment of the sample A is completed. In this case, the cartridge containing the sample A is removed, and thus the electrophoresis sample for which the CE analysis is not

started may be held in the standby unit 321, and the CE sample may be being migrated.

[0333]

ii) When two capillaries can be used for one sample,

FIG. 33 illustrates a typical example of an operation timing of the analysis system.

[0334]

Two CE units may be prepared for one sample analysis unit. In this case, it is preferred that the number of the CE units is twice that of sample processing units. In addition, the number of CE units may be equal to or larger than the number of a plurality of sample analysis units.

[0335]

As illustrated in (1) of FIG. 33, the electrophoresis sample m and the electrophoresis sample n may be subjected to the CE measurement in step 405 as soon as both samples m and n can be prepared, or as illustrated in (2) of FIG. 33, may start to be analyzed simultaneously in step 405 after both samples m and n are prepared. In addition, when the number of CE units is the same as the number of sample processing units and there is no space, the measurement may be performed in order from the electrophoresis sample m or the electrophoresis sample n.

[0336]

[Example 1]

The analysis system 101 includes one flow path device 104 and one CE unit 105.

[0337]

The forensic medicine sample containing an unknown amount of DNA is input to the dissolution chamber 301 (inlet for a sample or the like) of the flow path device 104. The processing of steps 401 to 403 is automatically performed within the flow path device 104.

[0338]

After m cycles of the PCR are performed in step 502, the amplification product of m

cycles is taken out in step 503. The sample is fed into the CE unit 105, and the CE measurement is started in step 504. In parallel with step 320, the PCR reaction solution remaining in the PCR chamber 304 is subjected to n cycles of the PCR in step 505. After the CE measurement in step 504 is completed and a next CE measurement can be started, the product of n cycles is fed into the CE unit 105 and the CE measurement is performed in step 507.

[0339]

[Example 2]

The analysis system 101 includes one flow path device 104 and two CE unit 105.

[0340]

The forensic medicine sample containing an unknown amount of DNA is input to the dissolution chamber 301 (inlet for a sample or the like) of the flow path device 104. The processing of steps 401 to 403 is automatically performed within the flow path device 104.

[0341]

After m cycles of the PCR are performed in step 502, the amplification product m is taken out from the PCR chamber 304 in step 503. The sample is fed into one side of the CE unit 105. In parallel with step 503, the PCR reaction solution remaining in the PCR chamber is subjected to n cycles of the PCR in step 505. In step 506, the product of n cycles is fed into the CE unit 105, and steps 504 and 507 are simultaneously started in a state in which the electrophoresis samples m and n are stored in the two capillaries.

Reference Signs List

[0342]

101: analysis system

102: computer

103: database

104: flow path device

105: CE unit

106: user interface

201 to 207: determination reference

301: dissolution chamber

302: purified film

303: purified film chamber

304: PCR chamber

305: waste liquid chamber

306: external connection port

307: pump and valve

308: PCR reagent storage unit

309: PCR reagent

310: electrophoresis reagent storage unit

311: electrophoresis reagent

312 to 314: reagent storage unit

315: flow path

316: flow path

317: heating unit

318: heating unit

319: flow path

320: dispensing chamber

321: standby unit

322: flow path

323 to 326: valve

327: mixing chamber

328 to 330: flow path

331: electrophoresis reagent storage unit

332 to 334: air storage unit

335: PCR reaction solution

336: electrophoresis sample

337 to 339: valve

401: sample input step

402: dissolution step

403: purification step

404: amplification step

405: detection step

701: liquid surface

702: vent filter

703: flow path

704: flow path resistance

705: liquid surface detection sensor

801: CE detection upper limit or an upper limit of the amplification product concentration at which the PCR can be accurately amplified

802: CE detection lower limit

803: analysis range of STR-CE

804: analysis range of STR-CE by split PCR

805, 806: plot

807: dynamic range of CE

CLAIMS

[Claim 1]

A DNA analysis system comprising:

a flow path device including a PCR chamber for performing a thermal cycle; and

a capillary electrophoresis unit configured to subject a PCR reaction solution to electrophoresis analysis, wherein

the DNA analysis system

stores values of m and n set in advance,

generates a first reaction solution by subjecting the PCR reaction solution to m thermal cycles in the PCR chamber,

takes out a part of the first reaction solution from the PCR chamber without changing compositions,

subjects the part of the first reaction solution to the electrophoresis analysis in the capillary electrophoresis unit,

generates, in the PCR chamber, a second reaction solution by subjecting the first reaction solution remaining in the PCR chamber to $n - m$ (where $n - m$ is an integer of 2 or more) thermal cycles such that a total number of thermal cycles is n ,

takes out at least a part of the second reaction solution from the PCR chamber without changing compositions, and

subjects the at least part of the second reaction solution to the electrophoresis analysis in the capillary electrophoresis unit.

[Claim 2]

The DNA analysis system according to claim 1, wherein

m is 20 or more and 32 or less.

[Claim 3]

The DNA analysis system according to claim 1, wherein
n is 24 or more and 36 or less.

[Claim 4]

The DNA analysis system according to claim 1, wherein
n is larger than m by 4 to 9.

[Claim 5]

The DNA analysis system according to claim 1, wherein
the DNA analysis system does not subject the part of the first reaction solution and
the at least part of the second reaction solution to analysis using a method other than the
electrophoresis analysis before the electrophoresis analysis.

[Claim 6]

The DNA analysis system according to claim 1, wherein
the DNA analysis system mixes at least one of the part of the first reaction solution
and the at least part of the second reaction solution with a solution containing a plurality of
DNA fragments.

[Claim 7]

The DNA analysis system according to claim 1, wherein
the DNA analysis system generates a mixed solution by mixing at least one of the
part of the first reaction solution and the at least part of the second reaction solution with pure
water, formamide, or a solution having a conductivity of 10 mS/cm or less before the

electrophoresis analysis.

[Claim 8]

The DNA analysis system according to claim 7, wherein
the DNA analysis system heats the mixed solution to 90°C or higher.

[Claim 9]

The DNA analysis system according to claim 1, wherein
the flow path device fully automatically performs a process from preparing the PCR
reaction solution to subjecting the PCR reaction solution to n - m thermal cycles.

[Claim 10]

The DNA analysis system according to claim 1, wherein
the flow path device includes a metering unit, and
the DNA analysis system meters a predetermined amount of the first reaction solution
within a range of 0.1% to 50% by transporting the first reaction solution to the metering unit
after the m thermal cycles are completed.

[Claim 11]

The DNA analysis system according to claim 1, wherein
the flow path device includes an openable and closable valve, and the part of the first
reaction solution is divided and taken out by closing the valve before the start of the m thermal
cycles and opening the valve after the completion of the m thermal cycles.

[Claim 12]

The DNA analysis system according to claim 1, wherein

the DNA analysis system maintains the part of the first reaction solution at a constant temperature in a range of 50°C to 80°C for 1 minute to 20 minutes.

[Claim 13]

The DNA analysis system according to claim 1, wherein
the DNA analysis system maintains the first reaction solution at a constant temperature in a range of 50°C to 80°C for 1 minute to 20 minutes, and then takes out the part.

[Claim 14]

The DNA analysis system according to claim 1, wherein
the DNA analysis system transports an entire amount of the PCR reaction solution in the PCR chamber to an outside of the PCR chamber at the completion of n thermal cycles.

[Claim 15]

The DNA analysis system according to claim 1, wherein
the PCR reaction solution includes two or more primer sets, and
the PCR reaction solution includes DNA including two or more amplified gene regions.

[Claim 16]

The DNA analysis system according to claim 1, wherein
the DNA analysis system
determines which of a result of the electrophoresis analysis on the part of the first reaction solution and a result of the electrophoresis analysis on the at least part of the second reaction solution is a better result, and
outputs the better result or outputs information capable of determining which

is the better result.

[Claim 17]

A DNA analysis system comprising:

a flow path device including a PCR chamber for performing a thermal cycle; and

a capillary electrophoresis unit configured to subject a PCR reaction solution to electrophoresis analysis, wherein

the DNA analysis system

stores values of m and n set in advance,

generates a first reaction solution by subjecting the PCR reaction solution to m thermal cycles in the PCR chamber,

takes out a part of the first reaction solution from the PCR chamber without changing compositions,

generates, in the PCR chamber, a second reaction solution by subjecting the first reaction solution remaining in the PCR chamber to $n - m$ (where $n - m$ is an integer of 2 or more) thermal cycles such that a total number of thermal cycles is n ,

takes out at least a part of the second reaction solution from the PCR chamber without changing compositions,

subjects one of the part of the first reaction solution and the at least part of the second reaction solution to the electrophoresis analysis in the capillary electrophoresis unit, and

controls execution of the electrophoresis analysis on another one of the part of the first reaction solution and the at least part of the second reaction solution based on a result of the electrophoresis analysis on the one of the part of the first reaction solution and the at least part of the second reaction solution.